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Germination of Cereal, Sorghum, and Small Legume Seeds After Fumigation with Hydrogen Phosphide[1]

R. G. STRONG and D. L. LINDGREN, *University of California Citrus Experiment Station, Riverside*

ABSTRACT

Tolerance of seeds of cereals, sorghums, and small legumes to hydrogen phosphide fumigation was tested under various conditions of moisture content of seeds, temperatures during fumigation, and dosages of fumigant. Representative seeds used were: cereals—California Mariout barley, DeKalb 459 corn, Kanota oats, Calrose rice, and Ramona 50 wheat; sorghums—Double Dwarf No. 38 milo, and Piper Sudan grass; small legumes—Ranger alfalfa, alsike clover, ladino clover, Kenland red clover, and Viking Birdsfoot trefoil. The moisture content of seeds approached the desired graded range of 9%, 11%, 13%, and 15% for cereals and milo; 8%, 10%, 12%, and 14% for Sudan grass; and 6%, 9%, and 12% for small legumes. Two dosages of fumigant, 9.0 and 18.0 mg. hydrogen phosphide per liter, were applied by placing weighed portions of aluminum phosphide tablets (Phostoxin) on top of cloth sample-bags of seeds in 28-liter fumigation chambers. Fumigation chambers were held in temperature-controlled cabinets at 50°, 70°, and 90° ± 2° F. for an exposure period of 7 days. One-half of the fumigated samples of seed received one fumigation; the others were fumigated twice. Germination of seeds was not impaired under the conditions of the study. It was concluded that it should be possible to use hydrogen phosphide for the control of storage pests in seeds of cereal grains, sorghums, and small legumes without injury to the germinative capacity of the seeds.

From the results of a series of experiments with aluminum phosphide tablets (Phostoxin[2]) to assess the efficacy and safety of the product, Heseltine & Thompson (1957) concluded that it should be possible to obtain good results using Phostoxin for the fumigation of grain in several types of storage: deep-bin silos, farm bins, and sacks; there should be no danger to health caused by its use provided reasonable precautions are taken, and there should be no damage to food grain treated with it. Ease of application would seem to be the main advantage of its use.

Experimental data presented by Lindgren *et al.* (1958) indicated that hydrogen phosphide evolved from aluminum phosphide tablets is effective as a fumigant against several species of storage pests, including the khapra beetle, *Trogoderma granarium* Everts. Germination of wheat was not impaired by fumigation with hydrogen phosphide in the limited tests conducted.

The effectiveness of hydrogen phosphide as a fumigant for the control of storage pests, the ease of application of aluminum phosphide tablets from which the fumigant is evolved, and the tolerance of wheat seeds to the fumigant suggested its potential usefulness for the control of insect pests in stored seeds. For this reason studies were initiated on the tolerance of seeds to hydrogen phosphide under various conditions of moisture content of seeds, temperatures during fumigation, and dosages of fumigant. It is the purpose of this paper to summarize results from germination tests with seeds of cereal grains, sorghums, and small legumes which were fumigated with hydrogen phosphide.

MATERIALS AND METHODS.—*Preparation of Seed Samples.*—The initial moisture contents of cereal, sorghum, and small legume seeds were: California Mariout barley—11.1%, DeKalb 459 corn—8.9%, Kanota oats—9.3%, Calrose rice—9.8%, Ramona 50 wheat—10.2%, Double Dwarf No. 38 milo—11.0%, Piper Sudan grass—9.8%, Ranger alfalfa—5.5%, alsike clover—9.1%, ladino clover—6.1%, Kenland red clover—6.6%, and Viking Birdsfoot trefoil—7.5%. The moisture content of cereal grains and milo was determined with a *Steinlite 400 G* moisture tester. Sudan grass seeds were ground to pass through a No. 20 sieve. Then well-mixed samples of about 2 grams were weighed accurately and dried at 130° ± 3° C. for 1 hour in a forced-air oven. Dried samples were weighed and the per cent loss in weight was calculated and considered as the moisture content of Sudan grass seeds. The moisture content of small legume seeds was considered to be the per cent loss in weight of 2-gram samples of whole seeds after drying for 1 hour in a forced-air oven at 130° ± 3° C.

Careful adjustment of moisture and conditioning of seeds was required to obtain samples approaching the desired graded moisture range of 9%, 11%, 13%, and 15% for cereals and milo; 8%, 10%, 12%, and 14% for Sudan grass; and 6%, 9%, and 12% for small legumes. Seeds were dried in narrow wire-screen containers placed in a forced-air oven maintained at 125° to 130° F. This temperature did not affect the germination of seeds which required drying. The drying period ranged up to 3 hours

[1] Paper No. 1170, University of California Citrus Experiment Station, Riverside California. Accepted for publication September 21, 1959. Partial cost of publication of this paper was met by the Hollywood Termite Control Co., Los Angeles, Calif.

[2] Phostoxin; manufactured by Degesch-Deutsch Gesellschaft für Schädlingsbekampfung, Germany. American supplier: Hollywood Termite Control Co.

Table 1.—Variations[a] in the percentage germination of fumigated from unfumigated seeds after one and two fumigations with hydrogen phosphide for 7 days at three temperatures and two dosages.

Kind and Variety of Seeds	No. of Fumigations	Moisture Content of Seeds (%)	Fumigation Temperatures and Dosages in Mg./L.					
			90° F.		70° F.		50° F.	
			9.0	18.0	9.0	18.0	9.0	18.0
Cereals:								
Barley, California Mariout	1	9.3	+1	−1	+2	+1	−3	−4
		11.1	0	−3	−2	−1	−2	−2
		12.8	−2	0	0	−1	−1	0
		14.7	+2	−2	+3	+3	−3	0
	2	9.3	+1	+1	−1	+1	+3	+2
		11.1	−1	−2	−1	−1	+1	0
		12.8	+1	+2	−3	0	+2	+2
		14.7	+1	+2	+1	+1	+2	+1
Corn, DeKalb 459	1	9.0	+1	+1	−1	−2	−1	−1
		10.8	−1	−1	0	+1	−2	0
		12.8	−1	−1	0	−2	+2	+3
		15.0	−1	−1	−4	+2	+2	0
	2	9.0	−1	+1	−1	−1	+1	0
		10.8	−1	+1	+1	−1	−1	−2
		12.8	+2	0	−3	−2	−4	−2
		15.0	+1	−1	+2	−2	−2	−2
Oats, Kanota	1	8.9	−1	0	−3	−1	−2	0
		11.0	−2	0	−2	+1	0	−2
		12.7	−1	+1	−4	−3	0	+2
		14.7	−2	−2	−1	+2	0	+1
	2	8.9	−3	−7	0	−1	−3	+1
		11.0	+2	+2	+3	+4	−1	−2
		12.7	+1	+1	+2	+4	+4	−2
		14.7	+9	+5	−2	+1	+1	−3
Rice, Calrose	1	9.3	+11	+2	+6	0	−2	+5
		10.8	−6	−10	−4	−7	0	+3
		13.2	−1	−6	+4	+4	−7	−5
		15.3	−3	−1	−4	0	+3	−5
	2	9.3	−3	−10	−6	−11	+5	+3
		10.8	+7	−11	−3	−4	−2	−2
		13.2	−5	−11	+4	−3	+8	+7
		15.3	−4	−9	−4	−7	−3	+1
Wheat, Ramona 50	1	9.2	+1	+1	−1	−3	0	+1
		10.8	+2	+3	−4	−1	+4	+1
		12.9	+1	+3	+3	+2	+3	+5
		14.7	+3	−2	−3	−2	+4	+3
	2	9.2	+1	−2	−1	−3	−2	−4
		10.8	+1	+2	+3	+2	+1	0
		12.9	+3	+1	−1	+1	−1	−2
		14.7	0	+3	+2	+2	−1	−2
Sorghums:								
Milo, Double Dwarf No. 38	1	8.6	+6	+9	−4	−3	−3	+1
		11.0	−4	−5	−3	−3	−1	+1
		12.9	−5	−4	−5	−5	+8	+4
		14.9	+5	+4	−2	+1	+4	−2
	2	8.6	+2	−2	−1	0	−3	−8
		11.0	+4	−8	−1	−7	+3	+3
		12.9	+2	−4	−3	−8	+4	−1
		14.9	−1	−5	+6	−4	−1	−1
Sudan grass, Piper	1	7.8	−1	+2	−1	−4	−1	+1
		9.8	−4	−5	−1	0	+3	+5
		12.3	−1	−2	−4	−4	−1	−1
		14.0	−1	−2	−3	−5	+5	+2
	2	7.8	0	−1	−3	−1	−7	−5
		9.8	−3	−1	−3	+3	+2	+7
		12.3	+1	−1	+4	+1	+3	+2
		14.0	−3	−2	+1	+1	−3	0
Small Legumes:								
Alfalfa, Ranger	1	5.8	−1	−2	−4	+2	+2	+1
		8.9	−6	−5	+2	−3	+1	−2
		12.0	+2	−4	+2	−2	+1	+2
	2	5.8	+2	+2	+6	+5	+1	+3
		8.9	+1	−2	0	−4	+2	+2
		12.0	+1	+4	−2	+2	+3	+3

[a] Mean differences in the percentage of normal seedlings in germination tests with fumigated seeds from corresponding tests with unfumigated control samples + indicates a greater, − less, and 0 the same percentage of normal seedlings in the fumigated as in unfumigated controls.

Table 1.—(Continued)

Kind and Variety of Seeds	No. of Fumigations	Moisture Content of Seeds (%)	Fumigation Temperatures and Dosages in Mg./L.					
			90° F.		70° F.		50° F.	
			9.0	18.0	9.0	18.0	9.0	18.0
Clovers:								
Alsike	1	6.2	+2	+2	0	+6	−3	−5
		9.1	+8	+14	+1	−1	−4	−4
		12.2	+2	+2	+2	+2	+6	+6
	2	6.2	+12	+7	+4	+2	+4	−3
		9.1	+6	+5	+1	+1	+3	+9
		12.2	−1	+4	−8	−5	−7	+4
Ladino	1	6.1	+1	−3	+3	−1	−7	−3
		9.3	+1	−1	+3	+3	−1	−3
		11.7	−7	−3	−6	−3	−3	−6
	2	6.1	+2	+2	−1	0	+1	+4
		9.3	+2	+1	+2	−3	−3	−6
		11.7	−6	−4	−4	+2	−7	+1
Kenland Red	1	5.9	−2	+1	+1	−2	0	−1
		8.8	+2	+1	+4	+2	−5	−1
		11.8	+3	+4	+1	−1	−1	−1
	2	5.9	+3	+3	−1	−5	+5	+2
		8.8	+4	+1	−3	−1	−2	−2
		11.8	+1	+1	−2	−1	+4	+4
Trefoil, Viking Birdsfoot	1	5.8	+4	−6	−3	−8	−4	−6
		9.1	+2	+6	+3	+2	+2	+3
		11.7	−5	−2	+1	−4	−1	−3
	2	5.8	−4	0	0	+3	+2	+8
		9.1	+5	+6	−5	−7	+4	+5
		11.7	+2	+1	−2	−2	−1	0

at any one time, but never longer. The moisture content of seeds before drying and the desired moisture level indicated the length of time seeds were to be held in the air-oven. Water was added to increase the moisture content as needed. After drying or addition of water, seeds were conditioned in airtight polyethylene bags or sealed metal containers for 7 days at 50° F. Seeds were then blended and moisture tests were made to determine the need for additional moisture adjustments.

When the moisture content of the seeds approached the desired level (see table 1 for actual moisture of seeds used), samples were placed in small rough-woven cotton bags. Seeds were removed from these bags only for germination tests. Each cloth sample-bag of seeds was placed in an airtight polyethylene bag to prevent moisture changes. Prepared samples awaiting fumigation were held in storage at 50° F.

Fumigation of Seeds.—The fumigation chambers used were of 28-liter volume without circulation. Cloth sample-bags of seeds placed on the bottom of each chamber filled approximately one-third of the space provided. Seeds of all moisture levels were placed inside the fumigation chambers to be fumigated together.

The fumigant was applied at two dosage levels: (1) 9.0 mg. hydrogen phosphide per liter (equivalent to approximately 10 Phostoxin tablets per ton of grain), and (2) 18.0 mg. hydrogen phosphide per liter (equivalent to approximately 20 Phostoxin tablets per ton of grain). Dosages were obtained by cutting Phostoxin tablets to two sizes, $\frac{1}{4}$ and $\frac{1}{2}$, weighing portions of tablets to the closest 0.01 gram of weight desired, and placing them in open petri dishes on top of the seeds in the fumigation chambers. The fumigation chambers were placed in temperature-controlled cabinets at 50°, 70°, and 90° ± 2° F. for an exposure period of 7 days. The exposure was measured from the time the chamber was closed after placing Phostoxin inside. No attempt was made to determine the length of time taken for gas evolution, nor to measure the concentration of gas in the chambers. Fumigations were replicated twice.

All seeds received an initial fumigation; one-half of the samples were fumigated a second time 4 weeks after the initial exposure to test the effect of repeat fumigation. Seeds were aerated for at least 24 hours after fumigation before use in germination tests. Seeds to be fumigated twice were aerated for 24 hours, then repackaged in airtight polyethylene bags and held in storage at 50° F. until ready for use in the second fumigation.

Germination Tests and Assessment of Results.—Germination tests were made within 10 days following fumigation. Seeds were germinated as follows: barley, oat, and wheat at 68° F. in folded-paper-towel substrata; milo and Sudan at alternating temperatures of 68° and 86° F. in folded-paper-towel substrata; corn and rice at alternating temperatures of 68° and 86° F. in upright rolled-paper-towel substrata; and the small legumes at 68° F. between blotters.

Germination tests and seedling interpretations were made in accordance with regular methods of Official Seed Analysts (Assoc. Offic. Seed Analysts 1954) except seeds with obvious mechanical injury were not planted, and 100 seeds were used per test for each treatment replicate. Unfumigated seeds served as control standards for comparison.

The percentage germination (normal seedlings) and the percentage of live seeds (normal and abnormal seedlings)

were the criteria recorded for comparison of results. Hard seeds were excluded in seedling counts of small legumes. Seedling interpretations were based on descriptions and illustrations given in the U. S. Department of Agriculture, Agriculture Handbook No. 30 (PMA and Bur. of Plant Industry, Soils, and Agricultural Engineering 1952).

RESULTS AND CONCLUSIONS.—A summary of results from germination tests is given in table 1 as the mean variations in the percentages of germination of fumigated seeds from unfumigated control samples. The small variations found between fumigated and unfumigated seeds were similar to variations between control samples. The differences between percentages of germinated (normal seedlings) and live seeds (normal and abnormal seedlings) were negligible; thus, only normal seedlings were considered in presenting the summary of results in table 1. It is obvious from these data that germination of cereal, sorghum, and small legume seeds was not impaired by hydrogen phosphide fumigation under the conditions of the study.

Although only one variety was tested for each kind of seeds used, this was representative of a wide range of varieties. Also, dosages of fumigant applied were high; one dosage, 18.0 mg. hydrogen phosphide per liter (equivalent to approximately 20 Phostoxin tablets per ton of grain), was extremely high. Thus, it is concluded that it should be possible to use hydrogen phosphide for the control of storage pests in seeds of cereal grains, sorghums, and small legumes without injury to the germinative capacity of the seeds.

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Control of the Northern Fowl Mite, *Ornithonyssus sylviarum* (C. & F.), with Ronnel, Bayer L13/59 and Bayer 21/199[1,2]

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ABSTRACT

Five hundred ninety-four hens and 100 roosters were used to test three organic phosphorous insecticides for control of the northern fowl mite, *Ornithonyssus sylviarum* (C. & F.). Bayer 21/199 (*O*-(3-chloro-4-methylumbelliferone, *O,O*-diethyl thiophosphate) as 0.5 and 0.25% dusts and Bayer L13/59 (*O,O*-dimethyl-2,2,2-trichloro-1-hydroxyethylphosphonate) and ronnel as 1% dusts were applied to individual birds on four body areas at the rate of 3 to 4 grams per bird. Each gave satisfactory control up to 28 days after treatment and apparently any one of the insecticides would virtually eradicate the mites if no birds were left untreated. No detectable residue could be found in the eggs of hens treated with Bayer 21/199 or ronnel and no off-flavor of eggs was detected.

The northern fowl mite, *Ornithonyssus sylviarum* (C. & F.), (Furman *et al.*, 1953) may exist unnoticed on poultry for some time and then increase to a severe infestation in a few days, reducing egg production in heavily infested birds. Many acaricides and insecticides have been tried for control. Vincent *et al.* (1954) reported a 4% malathion dust applied directly on the bird gave effective control. Furman & Coates (1957) reported that malathion exhibited many characteristics of the perfect insecticide for control, with a residual effect for approximately 30 days with either malathion dust or sprays. Rodriguez & Riehl (1958) also reported that malathion, as a residual spray, was effective. Linkfield & Reid (1958) reported that 4% and 5% malathion dusts failed to eradicate the mites but that 5% Trithion® (*S*-(*p*-chlorophenylthio) methyl *O,O*-diethyl phosphorodithioate) dust or 5% ronnel dust brought complete eradication for 8 or more weeks. There was no unfavorable egg taste or toxicity symptom in birds treated with either compound.

Hoffman (1956) reported Bayer L13/59 applied by sprinkling 3 grams of a 2.5% dust over the backs of birds controlled the mite 30 days after treatment. A 1% dust was ineffective. However, dusting with 5 grams of 5% dust of Bayer 21/199 or Bayer L13/59 gave a 100% control for 30 days.

In the current experiment, Bayer L13/59, Bayer 21/199, and ronnel were used as dusts for control of northern fowl mites on White Leghorns and Rhode Island Red chickens used for breeding experiments by the Poultry Husbandry Department of Kansas State University.

MATERIALS AND METHODS.—Polyethylene squeeze bottles were used to apply the dust to individual birds. All birds were from one infested house on the University's poultry farm. Each treated bird received four puffs from the polyethylene container, one under each wing, one around the vent and one above the preening gland. Ap-

[1] Contribution No. 748, Department of Entomology, Kansas Agricultural Experiment Station, Manhattan. Accepted for publication October 5, 1959. Partial cost of publication of this paper was met by the Chemagro Corporation, Kansas City, Mo.

[2] Ronnel [*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate], Dow Chemical Co., trademark Korlan®. Bayer L13/59 [*O,O*-dimethyl-2,2,2-trichloro-1-hydroxyethyl phosphonate], Chemagro Corp., trademark Dylox® and Dipterex®, licensed by Bayer Chemical Co. Bayer 21/199 [*O*-(3-chloro-4-methylumbelliferone, *O,O*-diethyl thiophosphate], Chemagro Corp., trademark Co-Ral®, licensed by Bayer Chemical Co.

[3] Assistant Instructor of Entomology.

Table 1.—Control of the northern fowl mite by various organic phosphorous dusts applied four different places on the bird's body during the winter of 1959, Manhattan, Kansas.

TREATMENT	DEGREE OF INFESTATION (AVERAGE RANK)[a]					
	Days Post-Treatment					
	0	3	7	14	21	28
Cage layers						
1% ronnel	5	0	0	0	1	0
1% Bayer L13/59	4	0	0	0	1	1
0.5% Bayer 21/199	5	0	0	0	0	0
0.25% Bayer 21/199	4	0	0	0	0	0
Inert dust only	3	2	2	2	2	2
Control	4	3	3	4	3	3
Floor pens						
1% ronnel	2	0	0	0	1	1
1% Bayer L13/59	2	1	0	0	1	1
0.5% Bayer 21/199	2	0	0	0	0	1
0.25% Bayer 21/199	4	0	0	0	1	1

[a] Criterion for determining infestation: 0, no mites seen; 1, less than 10 mites seen; 2, light infestation; 3, medium infestation; 4, heavy infestation; 5, very heavy infestation.

proximately 3 to 4 grams of dust were applied to each bird. One person did all of the treating.

The house contained one double layer of pens on each side with 10 hens and one cock in each pen. Two rows of double layer caged laying hens were in the middle. A total of 694 birds, 100 cocks and 594 hens, was treated as follows:

0.25% Bayer 21/199	114 hens, 22 cocks
0.5% Bayer 21/199	112 hens, 24 cocks
1.0% Bayer L13/59	123 hens, 15 cocks
1.0% ronnel	128 hens, 22 cocks
Inert dust (checks)	51 hens, 7 cocks
Untreated	66 hens, 10 cocks

Center caged birds of upper and lower tiers were divided into sections. Only centers of each section were counted to provide buffers between treatments.

Treatments were assigned to side pens so that the same treatment was not used in an adjacent pen. The only untreated control birds were in the two middle rows of caged layers.

Following dusting, a 0.25% Bayer 21/199 spray was applied on catch pans under the caged layers, end walls of the building, and pathways between cages to prevent mite migration. Extremely low pressure was used to minimize drift.

Half of the birds were examined for mites in each treatment at 0, 3, 7, 14, 21, and 28 days after treatment. The same person made all mite counts except on the 21st day. A numerical score, following Linkfield & Reid (1958), was used (see footnote, table 1).

A dozen eggs were collected from hens treated with Bayer 21/199, ronnel and their controls at each mite count and also at 35 days following treatment. The whites and yolks were frozen and stored until shipped to the respective chemical company for residue analyses.

RESULTS AND DISCUSSION.—Results (table 1) are summarized on the basis of infestation ranks. Treatments that gave longest residual control were assumed to be superior.

In the cage layer trials Bayer 21/199 at 0.25% and 0.5% gave excellent control over the period studied. Ronnel, at 1%, also gave very satisfactory results, with only one light infestation observed on one bird. This may have been a reinfestation shortly before counting since this bird was not visibly infested 7 days later.

All cages were subject to reinfestation to some extent, because egg gatherers and the experimenters went from cage to cage. Indications are, however, that any of the compounds used as a complete treatment would virtually eradicate the mites.

The 0.5% Bayer 21/199 dust gave the best control in the trial in the floor pens. There was little difference in effectiveness between the 0.25% Bayer 21/199 and the 1% ronnel or Bayer L13/59.

Chemical analyses of Bayer 21/199 residue in the eggs were done by Chemagro Corporation of Kansas City, Mo., using a photofluorometric method sensitive to 0.02 p.p.m. Chemical analyses of ronnel residue were performed by the Dow Chemical Company of Midland, Mich., using a manometric fly head cholinesterase inhibition technique sensitive to 0.05 p.p.m. No detectable residue was found in the eggs from the Bayer 21/199- or ronnel-treated hens.

Taste tests by several persons, including the senior author, indicated no difference in flavor between eggs from the treated or untreated hens.

ACKNOWLEDGMENTS.—Thanks are due Dr. James Craig and Amos Kahrs of the Department of Poultry Husbandry, Kansas State University, for their cooperation; to Dr. Herbert Knutson for advice; and to Stuart Ratcliff for generous assistance.

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Selective Toxicity and Animal Systemic Effectiveness of Several Organophosphates[1]

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Abstract

The toxicity of 17 organophosphates to rats varied with the substituents surrounding the phosphorus atom and the route of administration. Compounds with the highest rat/house fly toxicity ratios were dimethoate, Dibrom®, (*O,O*-dimethyl-*O*-(1,2,-dibromo-2,2-dichloroethyl) phosphate), and Bayer 29493 (*O,O*-dimethyl (*O*) 4-methyl-mercapto-3-methylphenyl phosphorothioate). Dimethoate, Bayer 29493, Bayer 23453 (*O,O*-dimethyl *S*-2-(ethylsulfinyl)ethyl phosphorodithioate), Bayer 24498 (*O,O*-dimethyl *S*-(methylsulfinyl)ethyl phosphorothioate), Bayer 25198 (*O,O*-dimethyl *O*-*p*-methylsulfinylphenyl phosphorothioate), and Shell SD 3562 (3-(dimethoxyphosphinyloxy)-*N,N*-dimethylcrotonamide) administered orally to rabbits were effective as animal systemics against the bed bug (*Cimex lectularius* L.) and Gulf Coast tick (*Amblyomma maculatum* Koch). The residual effectiveness (complete kill of test arthropods) of the systemically active esters was: dimethoate and Shell SD 3562, 2 hours, Bayer 24498, 4 hours; Bayer 25198, 6 hours; Bayer 23453 and Bayer 29493, 14 hours.

The effectiveness of organophosphates as chemotherapeutic agents has stimulated the empirical screening of compounds against ectoparasites feeding on laboratory animals prior to evaluation of a candidate material under field conditions. Since Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) injected subcutaneously and Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) given orally to cattle were effective against both species of cattle grubs, *Hypoderma lineatum* (DeVill.) and *H. bovis* (L.) (McGregor *et al.* 1954), the screening of organophosphates was intensified using rabbits (Adkins *et al.* 1955, Flynn & Eden 1958), guinea pigs (Drummond 1958) and mice (Hewitt *et al.* 1958a) as host animals and various species of insects and ticks as test arthropods. Practical control of the cattle grub under field conditions was accomplished with ronnel (Dow ET-57) administered orally (Adkins 1957, McGregor & Bushland 1957) and Bayer 21/199 (Co-Ral,® *O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) applied dermally (Brundrett *et al.* 1957). Two other organophosphates, which appeared promising as animal systemics in laboratory and field experiments, were dimethoate (Hewitt *et al.* 1958a, 1958b) and Dowco 109 (*O*-(4-tert-butyl-2-chlorophenyl) *O*-methyl methylphosphoramidothioate) (Roth & Eddy 1959).

The toxicity of several organophosphates administered orally to rabbits, orally and subcutaneously to white rats and topically to the house fly (*Musca domestica* L.) and the animal systemic activity of the compounds against the bed bug (*Cimex lectularius* L.) and Gulf Coast tick (*Amblyomma maculatum* Koch) feeding on rabbits treated orally are reported in this paper.

Procedure.—*Materials.*—The organophosphates included in this study were of technical grade and were used as received from the manufacturer. The chemical definition of each compound listed in table 1 is as follows:

Dibrom®, *O,O*-dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl) phosphate

Dimethoate, *O,O*-dimethyl *S*-(*N*-methyl-carbamoylmethyl) phosphorodithioate

Bayer 16948, *O,O*-diethyl *O*-2-(sec. butoxymethylthio)ethyl phosphorothioate

Bayer 18510, *O,O*-dimethyl *S*-(phenyl) (carboethoxy)methyl phosphorothiolate

Bayer 18779, *O*-ethyl-*O*-isopropyl-*O*-phthaloximido phosphorothioate

Bayer 22684, *O*-methyl-*O*-(2-chloroethyl)-*O*-(2-dichlorovinyl) phosphate

Bayer 22893, *O,O*-diethyl *O*-1,2,2-trimethylpropyl phosphorothioate

Bayer 23453, *O,O*-dimethyl *S*-2-(ethylsulfinyl)ethyl phosphorodithioate

Bayer 24498, *O,O*-dimethyl *S*-(methylsulfinyl)ethyl phosphorothiolate

Bayer 24869, *O*-ethyl-*O*-(β,β-dichlorovinyl-*O*-1-(methyl) (2-chloroethyl) phosphate

Bayer 24882, *O*-methyl-*O*-(β,β-dichlorovinyl)-*O*-1-(methyl) (2-chloroethyl) phosphate

Bayer 25198, *O,O*-dimethyl *O*-*p*-methyl sulfinylphenyl phosphorothioate

Bayer 29493, *O,O*-dimethyl (*O*)4-methyl-mercapto-3-methylphenyl phosphorothioate

Hercules 3895, 2,2-bis(ethylthio)vinyl diethylphosphate

Shell SD3562, 3-(dimethoxyphosphinyloxy)-*N,N*-dimethylcrotonamide

Trithion®, *O,O*-diethyl *S*-(*p*-chlorophenylthio)-methyl phosphorodithioate

UCC 8305, *P*-chloro-2,4-dioxa-5-methyl-*P*-thiono-3-phosphabicyclo(4.4.0) decane

Table 1.—Toxicity of several organophosphates to rats and the house fly.

Compound	LD50 Mg./Kg.			Toxicity Ratio	
	Rats				
	Oral	Subcutaneous	House Fly Topical[a]	Rat, Oral/Rat, Subcut.	Rat, Oral/House Fly
Dibrom	315	240	1.2±0.2	1.31	262.50
Dimethoate	425	275	1.3±0.3	1.55	326.90
Bayer 16948	23	55	32.0±2.6	0.42	0.72
Bayer 18510	130	45	15.5±2.6	2.89	8.38
Bayer 18779	115	160	26.2±3.4	0.72	4.38
Bayer 22684	80	38	2.4±1.0	2.11	33.33
Bayer 22893	110	425	44.0±5.1	0.26	2.50
Bayer 23453	235	425	80.0±7.7	0.55	2.93
Bayer 24498	22	12	39.0±7.8	1.83	0.56
Bayer 24869	48	40	4.7±0.8	1.20	10.21
Bayer 24882	75	32	3.7±1.1	2.34	20.27
Bayer 25198	8	21	5.0±1.3	0.38	1.60
Bayer 29493	325	450	4.8±0.9	0.72	67.70
Hercules 3895	10	18	6.8±1.6	0.56	1.47
Shell SD3562	45	50	45.0±7.7	0.90	1.00
Trithion	18	140	16.0±2.3	0.13	1.13
UCC 8305	85	80	23.5±3.6	1.06	3.62

[a] Confidence limits 5% level.

[1] Accepted for publication July 6, 1959.

Toxicity to Rats and House Flies.—Mixed sexes of white rats weighing from 175 to 225 grams each were used to determine the oral and subcutaneous LD_{50} values of the compounds shown in table 1. The compounds were diluted with corn oil and were administered orally through a 0.048-inch polyethylene stomach tube or subcutaneously with a 1-ml. syringe fitted with a 25-gauge needle. Dosage levels varied by a factor of 2 during preliminary toxicity studies. Rats were provided with food and water and held under observation for 48 hours after treatment. Twenty to 25 rats were used in each LD_{50} determination, which was estimated by gross inspection of the data.

Contact insecticidal activity to 3-day-old female house flies was based on 24-hour mortality counts following application of two time serial dilutions of the insecticide in acetone. House flies were anesthetized with carbon dioxide and 1 μl. of the insecticidal solution was applied to the abdomen with a microdosing device. Groups of 10 flies each were held for 24 hours after treatment in gauze-covered shell vials (25×95 mm.) containing a few grains of granulated sugar. Six to 10 replicates were treated, depending upon the variability of results. The LD_{50} values were calculated by the method of Litchfield & Wilcoxon (1949).

Animal Systemic Activity.—Procedures used for evaluating the animal systemic activity of insecticides were essentially the same as those described by Adkins *et al.* (1955) using rabbits as the host and bed bugs and Gulf Coast ticks as the arthropods. Three to five rabbits were treated orally with each compound in corn oil at each of the dosage levels given in table 2. About 25 fifth-instar bed bugs were placed on the shaved back of each rabbit 1 hour after treatment. Mortality counts were made 24 hours later on the bed bugs that fed. Gulf Coast ticks, about 25 nymphs, were allowed to attach to each rabbit 2 days prior to treatment; the mortality percentage was computed from the number that died on the host. In most instances, rabbits were treated at a sublethal dose as determined from rat toxicity data.

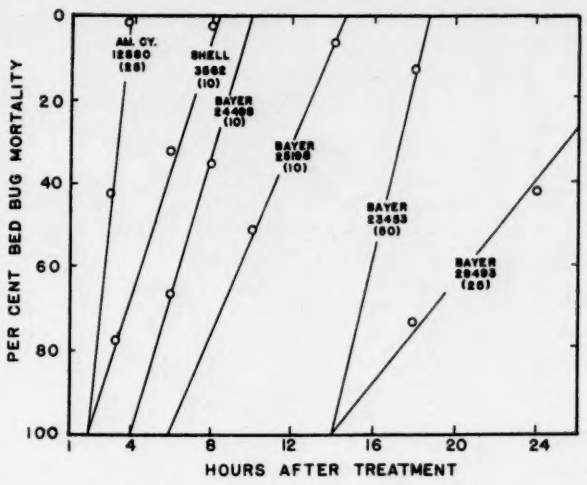

FIG. 1.—Residual systemic effect of several organophosphates administered orally to rabbits on the bed bug; numbers in parentheses indicate dosages in mg. per kg. to the rabbits (Am. Cy. 12880 = dimethoate).

Compounds exhibiting animal systemic activity against the bed bug and Gulf Coast tick were studied further to determine the residual systemic effectiveness against the bed bug. Three rabbits were treated orally with each of the materials at the dosage levels shown in figure 1. At 1, 2, 3, 4, 6, 8, 10, 14, 18, and 24 hours after treatment of the host, 15 fifth-instar bed bugs were allowed to take a blood meal from each rabbit. Mortality counts were made 24 hours after the bed bugs fed.

RESULTS AND DISCUSSION.—*Toxicity Studies.*—The compounds least toxic to rats (oral and subcutaneous LD_{50} values of greater than 200 mg. per kg.) were dimethoate, Dibrom®, Bayer 23453, and Bayer 29493, while the most toxic materials (LD_{50} values of less than 25 mg. per kg by either route of administration) were Bayer 24498, Bayer 25198, and Hercules 3895 (table 1). In most instances the route of administration greatly influenced toxicity of a compound to rats. Eight of the 17 compounds shown in table 1 were more toxic when administered orally (oral/subcutaneous toxicity ratio less than 0.8); 6 were more toxic when administered subcutaneously (toxicity ratio greater than 1.2); and 3 were about equally toxic by either route of administration (toxicity ratio between 0.8 and 1.2). Compounds that were more toxic to rats when given orally as compared with subcutaneously were all phosphorothioate or phosphorodithioate esters, and 5 of the 8 materials contained thioether or thiophenyl groups in the acid residue portion of the molecule. Although dimethyl and diethyl groupings were about equally represented in these eight compounds having greater oral than subcutaneous toxicity to rats, the greatest toxicity difference was with diethyl compounds (Bayer 22893 and Trithion). All compounds that were more toxic subcutaneously than orally to rats contained dimethyl groups; three were phosphates, two were phosphorothiolates, and one was a phosphorodithioate.

The descending order of toxicity of compounds with

Table 2.—Systemic insecticidal effectiveness of several organophosphates to bed bugs and ticks when administered orally to rabbits.

COMPOUND	NO. RABBITS TREATED	DOSE TO RABBITS (MG./KG.)	PER CENT MORTALITY[a]	
			Bed Bugs	Ticks
Dibrom	3	50	—[b]	—[b]
	5	25	9 (56)	0 (36)
Dimethoate	4	50	100 (72)	97 (66)
	3	25	100 (50)	40 (42)
Bayer 16948	3	10	0 (43)	0 (31)
Bayer 22684	3	25	0 (58)	0 (42)
Bayer 22893	3	25	0 (51)	0 (32)
Bayer 23453	3	50	100 (38)	86 (44)
	3	25	91 (44)	0 (39)
Bayer 24498	3	25	100 (48)	100 (53)
	4	10	100 (50)	64 (64)
	3	5	100 (41)	0 (43)
Bayer 24869	3	10	0 (44)	0 (46)
Bayer 24882	3	50	0 (71)	0 (48)
Bayer 25198	5	10	100 (74)	36 (42)
Bayer 29493	3	25	100 (45)	59 (39)
	3	10	0 (45)	0 (35)
Hercules 3895	3	10	4 (39)	14 (29)
Shell 3562	3	25	100 (46)	100 (51)
	3	10	100 (44)	72 (54)
Trithion	3	50	—[b]	—[b]
	3	25	0 (49)	0 (48)
UCC 8305	3	25	0 (49)	0 (37)

[a] Numbers of bed bugs and Gulf Coast ticks feeding on rabbits 1 hour after treatment are shown in parentheses.

[b] Rabbits died before mortality counts were made.

the highest rat (oral/house fly toxicity ratio were dimethoate, Dibrom, and Bayer 29493 (table 1). Bayer 16948 and Bayer 24498 were more toxic to rats (oral) than to house flies. Other compounds having rat (oral)/house fly toxicity ratios of less than 2.0 were Bayer 25198, Hercules 3895, Shell SD 3562, and Trithion.

Animal Systemic Studies.—Dimethoate, Bayer 23453, Bayer 24498, Bayer 25198, Bayer 29493, and Shell SD 3562 administered orally to rabbits at the dosages shown in table 2 were effective as animal systemics against the bed bug and Gulf Coast tick taking a blood meal from the treated animals. The bed bug was a more sensitive arthropod than the Gulf Coast tick in evaluating animal systemic activity. Bayer 23453, Bayer 24498, and Shell SD 3562 were not very toxic when applied topically to the house fly (table 1), but were quite effective as animal systemics (table 2). Toxicity differences between rats (table 1) and rabbits were also noted for some of the compounds: dimethoate at 50 mg. per kg. orally, 1 out of 4 rabbits died; Bayer 24498 at 25 mg. per kg. orally, 2 out of 3 rabbits died; and Bayer 25198 at 10 mg. per kg. orally, 1 out of 5 rabbits died. The greatest difference in the toxicity of a single compound administered orally to different mammals was with Dibrom, which had a rat LD_{50} of 315 (oral) but 3 out of 3 rabbits died when treated at 50 mg. per kg. (oral). With most of the compounds, the rat toxicity data served as a guide in selecting a safe dosage level for treating rabbits.

The residual systemic effectiveness of these phosphorus esters are shown in figure 1. When bed bugs were fed on rabbits at 1, 2, 3, 4, 6, 8, 10, 14, 18, and 24 hours after treatment dimethoate and Shell SD 3562 gave 100% kill for 2 hours; Bayer 24498, 4 hours; Bayer 25198, 6 hours; Bayer 23453 and Bayer 29493, 14 hours.

All of the compounds exhibiting animal systemic activity contained dimethyl groupings and all except Shell SD 3562 were phosphorodithioates,—thiolates or—thioates. Dimethoate and Shell SD 3562 are similar in that both have alkyl-carbamoyl groups; Bayer 23453, Bayer 24498, Bayer 25198, and Bayer 29493 are similar in that each contains thioether or thiophenyl groups. The importance of the alkylcarbamoyl group (Hewitt *et al.* 1958a, Flynn & Eden 1958) and the thioether group (Adkins *et al.* 1955, Drummond 1958) in animal systemic effectiveness have been reported.

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Drosophila Breeding in Refuse from Tomato Canneries[1,2]

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Abstract

Surveys were made on the Eastern Shore of Maryland to determine the amount of drosophila breeding in refuse from tomato canneries as influenced by methods of disposal. Very little breeding occurred when the refuse was spread thinly over a field with a manure spreader. Refuse dumped in truckload piles or pumped from the cannery to a shallow pit was found to be a source of infestation for nearby canneries and fields. *Drosophila melanogaster* (Meig.), *D. busckii* (Coq.), D. *hydei* (Sturt.), and D. *transversa* (Fall) were found breeding in the refuse.

Tomato processors in the major tomato-growing areas of the United States have long recognized drosophila as a serious problem. They are known to breed in large numbers in certain cull fruits and vegetables discarded in piles or as culls scattered on the ground in vegetable fields or orchards (Bickley & Ditman 1954, Dorst & Knowlton 1954, Pepper *et al.* 1953). According to Michelbacher *et al.* (1953), melon fields and certain fruit orchards are

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[2] This investigation was made in cooperation with the Maryland Agricultural Experiment Station and the following canneries in Maryland: T. Noble Jarrell, Inc., Goldsboro; Logan-Ford Canning Co., Henderson; Salem Packing Co., Salem; Walter T. Andrews and Son, Cambridge; Phillips Packing Co., Inc., Vienna; and Harrison and Jarboe, Easton. W. E. Bickley and L. P. Ditman, of the Department of Entomology, University of Maryland, offered many helpful suggestions and assisted in making the initial contacts with some of the canners.

[3] W. N. Smith and B. K. Dozier, formerly of the Entomology Research Division, assisted in these surveys.

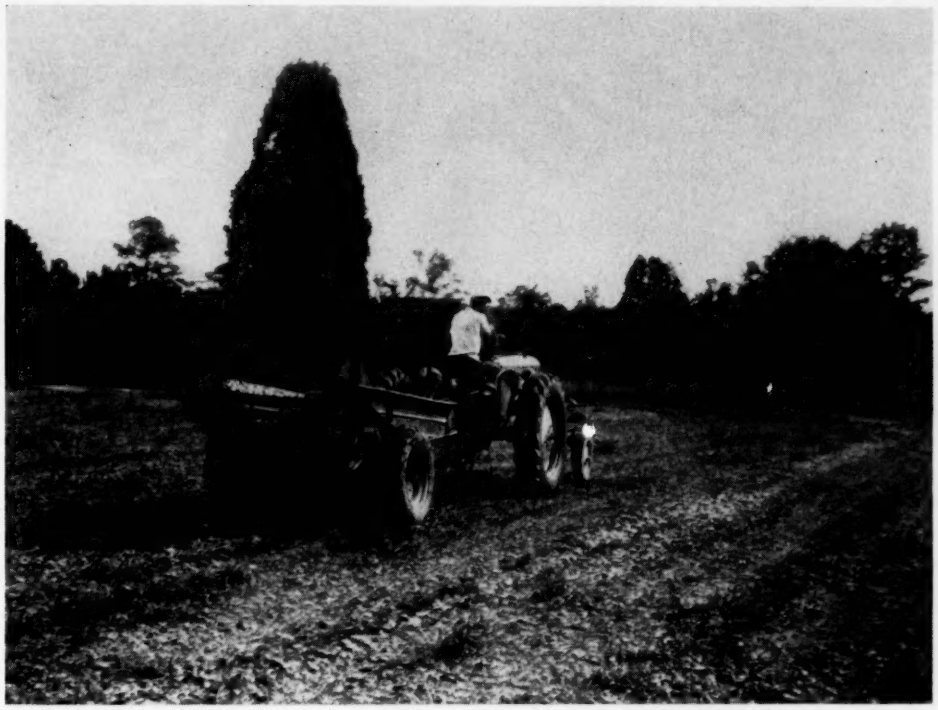

FIG. 1.—Tractor-drawn manure spreader used for scattering tomato refuse.

sources of infestation for tomatoes that grow adjacent to them.

In 1953, 1954, and 1955 surveys were made to determine the degree of drosophila breeding in refuse from tomato canneries on the Eastern Shore of Maryland. Observations were made from the last of August until October or November, the period when this problem is most serious. The refuse and surrounding areas were carefully examined. Occasionally refuse containing larvae and pupae was collected and held in the laboratory for adult emergence to determine the predominant species.

METHODS.—Observations were made in refuse or skins disposed of by three methods: (a) thinly spread over a field with a manure spreader (fig. 1), (b) dumped from a truck or trailer in scattered piles over a field (fig. 2), and (c) pumped through a pipe into a shallow pit near the cannery (fig. 3). The purpose was to ascertain, first, which method was the most effective for the prevention of drosophila breeding, and second, if sufficient breeding occurs in refuse from tomato canneries to be an important source of infestation for nearby canning factories and tomato fields.

Refuse Thinly Spread.—Only one canner in the area was using this method of disposal during the period of this study. A field of about 4 or 5 acres was sufficient for his plant, as the refuse dried soon after it was scattered and the operator discarded over the same area many times during the season.

This method was very effective. Almost no breeding occurred in any of the three seasons, probably owing to the rapid drying of the skins. In 1953 the infestation of adults in the field was very light, and only a few larvae and pupae were found at a time. In 1954 more adults were observed in the field than in 1953, and they were fairly numerous by the last of September. However, breeding, was very light all season except on October 11 in one area of about 6 to 8 square feet. This area was low and the skins had not dried because of poor drainage. In 1955 it was about the same. Light breeding was observed on September 7 and 19, but was limited to wheel ruts in low areas where drainage had been poor. The last day of canning for this factory was September 18 in 1953, October 5 in 1954, and September 7 in 1955.

Refuse in Scattered Piles.—Skins disposed of in this manner spread considerably over the ground when dumped, owing to the large amount of water in the refuse. Many of the piles were as much as 10 feet in diameter, but few were more than 12 inches deep.

Three fields were used for observations in 1953, and one field in 1954 and 1955. The fields were also used for grazing cattle. The refuse was fed upon by cattle, and some of the piles were severely trampled and mixed with soil.

In 1953 drosophila breeding was heavy by the latter part of September in piles that had not been heavily fed upon or severely trampled by cattle. Adults and eggs were not observed in large numbers before September 18. In 1954 breeding was light in refuse disposed of by this manner. By September 8 adults and eggs were numerous in some piles. After this date sufficient adults were present for heavy breeding had conditions been favorable. Breeding was very heavy in two truck loads of tomatoes within a few days after they had been accidentally overturned along the highway. One was overturned about September 1 near Cambridge, and the other about September 29 near Easton. Light breeding in the small piles of refuse is be-

FIG. 2.—Dumping tomato refuse from a truck in scattered piles over a field.

lieved to be due largely to severe trampling by grazing cattle. In 1955 the amount of breeding was about the same as in 1954. Adults were less numerous, but the light breeding was probably due mainly to grazing cattle. In 1953 and 1954 canning was discontinued about October 1 and in 1955 about September 1.

Refuse in Pits.—The refuse pits at some of the canneries were very shallow and soon overflowed. By the end

FIG. 3.—Pumping tomato refuse from a canning factory through a pipe to a nearby shallow pit.

FIG. 4.—Removing tomato refuse from shallow pit at end of canning season.

of the canning season some skin piles were about 40 feet in diameter and 4 feet deep. The refuse was normally removed from the pits before the start of another canning season (fig. 4). Observations were made at three canneries using this method of disposal in 1953, and at two in 1954 and 1955.

In 1953 there was very little drosophila breeding in the pits during the canning season, but heavy breeding was observed at all canneries within 8 to 14 days after canning ceased. After September 14 sufficient adults were present for heavy breeding had conditions been favorable. During most of the season there were seldom breaks of more than 2 days in the canning schedule. The frequent addition of refuse is believed to be responsible for the light breeding in the latter part of the season. The mixture of refuse and water over the top of the pile destroyed eggs, larvae, and pupae. Processing was discontinued between September 15 and 30.

In 1954 adults were numerous around both pits on September 1, and the population remained high during the rest of the canning season. In one pit breeding was light and scattered until after the plant closed, about September 15, and then was very heavy, as the last skins discarded were heavily infested with pupae on September 23 and 30. In the second pit heavy breeding was observed in part of the pile on September 30, but this was an area that had not been overflowed by refuse from recent operations. On October 26 breeding was heavy in skins dumped the last day of canning, about October 15.

In 1955 breeding was heavy at both pits on September 7 in skins that were a few days old. Larvae and pupae were numerous, but the adult population was not high. Breeding continued to be heavy during the remainder of the season. One canner stopped processing on September 15 and the other on October 19. Both operated on an irregular schedule during most of the season and were often closed for several days at a time.

SPECIES FOUND IN REFUSE.—Collections of larvae, pupae, and adults from tomato refuse in 1953 and 1954, showed the following species of *Drosophila* to be prevalent at times: *melanogaster* (Meig.), *busckii* (Coq.), and *hydei* (Sturt.). *D. melanogaster* and *hydei* were most common in 1953. All three species were numerous in 1954. A few specimens of a fourth species, *transversa* (Fall), were collected in 1954, but it was never as prevalent as the other three.[4]

CONCLUSIONS.—It was concluded that the most effective method of refuse disposal for the prevention of drosophila breeding is the scattering of skins thinly over a field. In refuse disposed of in piles or pits, sufficient breeding may occur to be a source of infestation for nearby canneries and tomato fields.

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[4] All species identified by W. W. Wirth, of the Entomology Research Division.

Laboratory and Field Tests Against Mites and Lice Attacking Poultry[1]

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ABSTRACT

In laboratory tests against the northern fowl mite *Ornithonyssus sylviarum* (C. & F.), ronnel (Korlan) was more toxic than malathion or barthrin. In spray tests on infested birds, dicapthon and ronnel were more effective than malathion, barthrin, or diethyltoluamide. The chicken mite *Dermanyssus gallinae* (DeG.) was far more difficult to kill than the northern fowl mite. In field tests on turkeys infested with the northern fowl mite and on chickens infested with the chicken body louse *Menacanthus stramineus* (Nitz.), excellent results were obtained with both ronnel and malathion.

Laboratory research on methods for evaluating insecticides against mites and lice attacking poultry has been very limited. In view of the importance of these arthropods and the increasing number of candidate materials, a laboratory screening procedure should aid research considerably, especially in determining insecticidal resistance. A method is described which appeared to work satisfactorily for both mites and lice, although it was developed mainly for use against mites. Preliminary data are presented along with results of practical tests on individual chickens and on flocks.

EXPERIMENTS WITH MITES.—*Laboratory Methods.*—The northern fowl mite (*Ornithonyssus sylviarum* (C. & F.)) was used for working out a test method, since it is one of the more difficult species to confine. The beaker method of evaluating insecticides against the body louse *Pediculus humanus humanus* L. described by Eddy (1952) was found to work very well for lice on chickens but was not satisfactory for mites owing to the problem of keeping them confined. However, insecticide-impregnated cloth gave uniformly good results. White 80-mesh cotton percale was found to be effective, as the mites could be easily seen and the mesh size was sufficient to prevent escapes. Other types of cloth would no doubt work equally well.

The test equipment (fig. 1) consisted of a metal ring and band and two pieces of cloth. The ring fitted over the band with the cloth secured between them in a manner similar to the use of embroidery hoops. The inside diameter of the ring was approximately 2 inches, and the band was slightly smaller. The inside of the ring and outside of the band were turned on a metal lathe so that they were smooth and circular. The two contact surfaces were coned, or made at a slight angle so that the tightness of fit was determined by the pressure applied when one piece was fitted or placed over the other. The band was made from a 2-inch pipe. Approximately one-half inch was smoothed down and another one-half inch left for handling purposes. Although 3-inch-square pieces of cloth were used, circular die-cut pieces should prove more convenient. One piece was placed over the band and the mites, anesthetized with carbon dioxide, were placed in the center with a camel's-hair brush (fig. 2). The other piece was placed over the mites and the ring pressed down over the cloth, confining them between the two pieces.

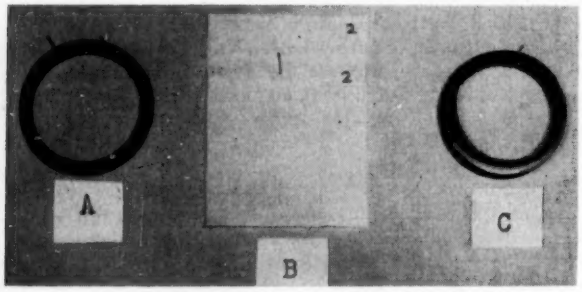

FIG. 1.—Mite-testing equipment: *A*, metal ring; *B*, two pieces cloth; *C*, metal band.

FIG. 2.—Transferring chicken mites anesthetized with carbon dioxide to treated cloth with camel's-hair brush.

The candidate insecticides were applied to the cloth as acetone solutions. The cloth patches were placed on a wire rack, and 0.65 ml. of the test solution was pipetted uniformly over each. This amount was sufficient to give good coverage without runoff. The treated patches were allowed to dry in the open for about an hour before being put in tests. During tests the ring-band-cloth setup was placed on edge, to reduce fumigation to a minimum. The tests were made at 78° to 80° F. and approximately 60% relative humidity.

Only adult female mites were used in the tests. In most of the initial experiments the mites (northern fowl) were collected from chickens that had died, apparently from an overload of mites. The chicken was placed inside a paper bag and a shell vial placed in the opening of the bag and tied securely. The vial was pointed toward a window, and since the mites are attracted to light, they soon found their way into the vial. Although carbon dioxide can be used for collecting mites or lice off either dead or live birds, a suction apparatus is preferred. The type described by Hoffman (1950) was used, since the suction could be varied to meet the needs for either mites or lice.

Laboratory Tests.—Data on the relative toxicity of barthrin, malathion, and ronnel (Korlan) to the northern fowl mite are presented in table 1. Malathion appeared to be slightly more toxic than barthrin, and ronnel was

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Table 1.—Toxicity of insecticides to the northern fowl mite in laboratory tests.[a]

INSECTICIDE	PER CENT CONCENTRATION	PER CENT MORTALITY AFTER 24 HOURS
Barthrin	0.01	10
	.025	15
	.05	65
Malathion	.01	20
	.025	30
	.05	95
Ronnel	.01	100
	.025	100
	.05	100
Check (acetone-treated)	—	0

[a] 3 replications, mites held 4 days before testing.

much more toxic than either. The relative susceptibility of the chicken mite *Dermanyssus gallinae* (DeG.) and northern fowl mite to malathion and ronnel is shown in table 2. The northern fowl mite is by far the more susceptible species. Ronnel was considerably more toxic than malathion to this mite, but there appeared to be little difference in insecticides in the case of the chicken mite.

Further tests were made to determine the minimum lethal concentration of ronnel against the northern fowl mite. At concentrations of 0.0005, 0.001, 0.0025, and 0.005% the mortality after 24 hours was 5, 18, 87, and 100%, respectively.

A few exploratory tests were conducted to determine the relative susceptibility to insecticides of nymphal and adult mites. Adult male chicken mites were approximately 20 times more susceptible to ronnel than adult females at the LD-90 level. Against the northern fowl mite ronnel was about 25 times more toxic to nymphs and males than to adult females at the LD-50 level. Malathion appeared to be only slightly more effective against northern fowl mite males and nymphs than to adult females at the concentrations used.

Table 2.—Susceptibility of the chicken and northern fowl mites to malathion and ronnel in laboratory tests.

INSECTICIDE	PER CENT CONCENTRATION	PER CENT MORTALITY AFTER 24 HOURS	
		Chicken Mite	Northern Fowl Mite
Experiment 1 (Mites tested on day collected)			
Malathion	0.005	70	60
	.01	85	90
	.025	80	100
	.05	60	100
Ronnel	.005	0	100
	.01	10	100
	.025	75	100
	.05	80	100
Check (acetone-treated)	—	0	0
Experiment 2 (Mites tested 4 days after collection)			
Malathion	0.005	20	0
	.01	55	37
	.025	50	68
	.05	70	100
Ronnel	.005	25	100
	.01	70	100
	.025	80	100
	.05	85	100
Check (acetone-treated)	—	0	5

FIG. 3.—Estimating mite infestation by use of wire loop.

Tests on Individual Chickens.—Estimating mite populations on chickens is rather difficult. Generally the infestations are classified as light, moderate, or heavy. In some of the initial tests the specimens were counted in five places—around the head and vent, on the breast, and under each wing. Later the size of the area was standardized by the use of a wire loop, two-fifths inch in diameter (fig. 3), and loop counts under the wings were discontinued in favor of the removal of five feathers from under each wing. Only adults and late-instar nymphs were included in the counts. It is believed that loop counts only on the breast and around the vent will work satisfactorily on both mites and lice.

The insecticides were formulated in the laboratory on a weight-per-volume basis as 25% emulsion concentrates, with Triton X-100 as the emulsifier and xylene as the solvent. They were applied with a DeVilbiss sprayer at a pressure of 40 p.s.i. The birds were hung up by one leg and rotated during the spraying to insure better coverage. They were allowed to dry before being placed in individual wire cages. Cage size was 16×16×16 inches and the mesh 1×2 inches. The cages were separated and kept from contact with the floor by suspending them from the ceiling rafters of a well-ventilated barn.

As shown in table 3, dicapthon and ronnel were more effective than malathion. Although rather erratic results were obtained with barthrin, it was less effective. Diethyltoluamide showed little promise.

Tests on Flocks.—Three field experiments were made against the northern fowl mite infesting turkeys. In the first experiment 12 moderately infested (45 to 100 mites per bird) adult gobblers were sprayed with a 0.25% ronnel emulsion, one-half pint being used per bird, and in the second experiment 30 turkey hens were sprayed with a 0.1% emulsion. Complete control was obtained through the fourth week, when the last examination was made.

Table 3.—Results of spray tests on the control of the northern fowl mite on individually caged birds.

Insecticide	Per Cent Concentration	Milliliters per Bird	Mites per Bird	Per Cent Control After (Days) 1	7	14	21
Malathion	0.05	25	82	44	2	41	66
	.1	40	22	64	100	100	—
	.5	25	111	100	100	100	100
Dicapthon	.05	40	28	100	100	100	100[a]
	.1	40	105	48	100	100	100[a]
Ronnel	.05	25	171	64	100	100	65
	.1	40	36	95	100	100	—
	.1	40	16	100	100	100	—
	.5	25	18	100	100	100	100
Barthrin	0.5	25	105	66	0	31	0
	.1	40	49	19	51	56	51
	.1	40	111	40	30	65	—
	.1	40	46	59	100	100	—
	.25	40	37	19	98	100	100
	.25	40	151	15	0	—	—
	.5	25	24	83	100	100	100[a]
Diethyltoluamide	.05	25	213	36	32	31	0
	.5	25	36	70	24	64	39
	2.5	40	88	96	95	100	—
	5.0	25	9	45	100	100	100[a]

[a] 100% also after 28 days.

The third experiment was made with 10- to 15-bird flocks of similarly infested turkey hens housed in the same building but in separate rooms, each with 168 square feet of floor space. In one room the birds, floor, and walls were treated with 2 gallons of a 0.5% malathion spray, and in another room they were treated with the same amount of ronnel. In the third room a 4% malathion dust was applied as a litter treatment at the rate of 1 pound to 40 square feet of floor space. All three treatments gave excellent control, as no live mites were found on the birds at weekly examinations made over a period of 2 months.

Experiments with Lice.—*Tests on Individual Chickens.*—The methods used for the control of the chicken body louse *Menacanthus stramineus* (Nitz.) were the same as those for the control of the northern fowl mite. The data are presented in table 4. Only one bird was treated at each concentration shown. Both malathion and ronnel gave complete control through 28 days at the low concentration of 0.05%. 3,4-Dimethylbenzyl chrysanthemumate appeared to be more effective than barthrin but less so than either of the phosphorus compounds. The data indicate that lice are easier to control than mites.

Table 4.—Results of spray tests on the control of the chicken body louse on individually caged birds.

Insecticide	Per Cent Concentration	Lice per Bird	Per Cent Control After (Days) 1	14	28
Malathion	0.05	57	100	100	100
	.1	62	100	100	100
Ronnel	.05	61	100	100	100
3,4-Dimethylbenzyl chrysanthemumate	.1	49	74	90	100
Barthrin	.1	67	63	69	53

Tests on Flocks.—Ronnel was tried as a litter treatment on a small flock of 13 hens infested with the chicken body louse. A commercial 5% dust was used at 1 pound to 40 square feet of floor space. No live lice were found on the chickens 2 and 4 weeks after treatment.

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The Penetration of Piperonyl Butoxide as a Synergist and as an Antagonist in *Musca domestica* L.[1]

George W. Ware[2]

Abstract

Penetration studies were made on 4-day-old male house flies (*Musca domestica* L.) with Sevin® (1-naphthyl-*N*-methyl carbamate) and piperonyl butoxide as a synergist, and malathion and piperonyl butoxide as an antagonist. Topical applications were made and groups of 100 flies were held for four time intervals, 6, 12, 18, and 24 hours, then extracted externally and internally. Companion treatments of piperonyl butoxide alone were extracted at those time intervals. The extracts were evaporated and the methylenedioxyphenyl color complex developed and read at 660 mμ. The rates of piperonyl butoxide penetration alone and with the toxicant were generally the same. A difference in the percentage of penetration and probably metabolism was observed between the two different levels of piperonyl butoxide, the lower level penetrating more rapidly percentagewise than the higher concentration. The penetration rate of piperonyl butoxide does not appear to determine its role as a synergist or antagonist.

Rai *et al.* (1956) demonstrated antagonism in the interaction of malathion and piperonyl butoxide. Ware & Roan (1957) demonstrated synergistic and antagonistic action of piperonyl butoxide with malathion and five of its analogs. The effect of the synergist seemed to depend on the structure of the malathion analog.

The investigations reported here were designed to

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[2] Assistant Professor, Department of Zoology and Entomology, Ohio State University, Columbus 10.

evaluate the effect of two choline esterase-inhibiting compounds on the penetration rate of piperonyl butoxide in the house fly, *Musca domestica* L.

MATERIALS AND METHODS.—Piperonyl butoxide,[3] 100%, was used in both synergism and antagonism penetration studies. Malathion, 99.6%, was used for the antagonism-penetration studies and technical grade Sevin®, (1-naphthyl-*N*-methyl carbamate) was used for the synergism-penetration studies.

Male house flies served as the test insect and were reared and treated topically under the conditions described by Ware & Roan (1957).

Petroleum ether, B. P. range 35–70° C., used in external and internal extractions, and acetonitrile, practical, Eastman Organic Chemicals, were both glass-distilled. Concentrated sulfuric acid, reagent grade, was used in all tests. Gallic acid, N.F., technical grade crystal, was purified by the method of Blum (1955).

The piperonyl butoxide color complex was developed by modification of that described by Blum (1955), and read at 660 mμ. on a Bausch & Lomb "Spectronic 20" equipped with the appropriate photo cell and filter.

The flies were treated on the mesosterna with acetone solutions of malathion and piperonyl butoxide (1:10, by weight) at 0.35:3.5 μg./μl. to obtain 40% to 50% mortality for antagonism penetration studies. Synergism studies utilized the same arrangement of Sevin and piperonyl butoxide at 0.125:1.25 μg./μl. Parallel groups of flies were treated with piperonyl butoxide alone for penetration comparisons at 3.5 and 1.25 μg./μl.

Four time intervals were selected for extractions: 6, 12, 18, and 24 hours following treatment. For each test 1,200 male flies were treated, 400 with piperonyl butoxide and 800 with piperonyl butoxide and the toxicant.

Standards were made from each piperonyl butoxide solution by collecting samples of 100 strokes of the microapplicator in 250-ml. flasks. These flasks were capped with aluminum foil and developed with the extractions.

Surviving flies were extracted externally with three 10-ml. portions of petroleum ether, which was filtered and dried. The internal extractions were made according to the method of Armstrong *et al.* (1952) except for the substitution of petroleum ether for cold methanol. Using this extraction method 95% recovery of the applied piperonyl butoxide was obtained. Untreated flies constituted the blank.

After drying, 5 ml. of acetonitrile and 50 mg. of gallic acid were added to each dried extract. The flasks were first heated (60° C.) until the acetonitrile was reduced to approximately 2 ml., then cooled and 12 ml. of sulfuric acid was slowly added down the sides of the flasks. After 1 minute the flasks were swirled to mix the layers. A green to blue-green color developed slowly and the optical density was read after 30 minutes.

RESULTS.—Five tests each were completed using piperonyl butoxide alone and with malathion. In the synergism pentration studies, six tests each of the compound alone and with Sevin were completed.

Figures 1 and 2 reveal a more rapid external disappearance of piperonyl butoxide alone than when it accompanies either toxicant. In both cases more remains externally with the toxicants at the 6- and 12-hour intervals and at an 18-hour interval with malathion than

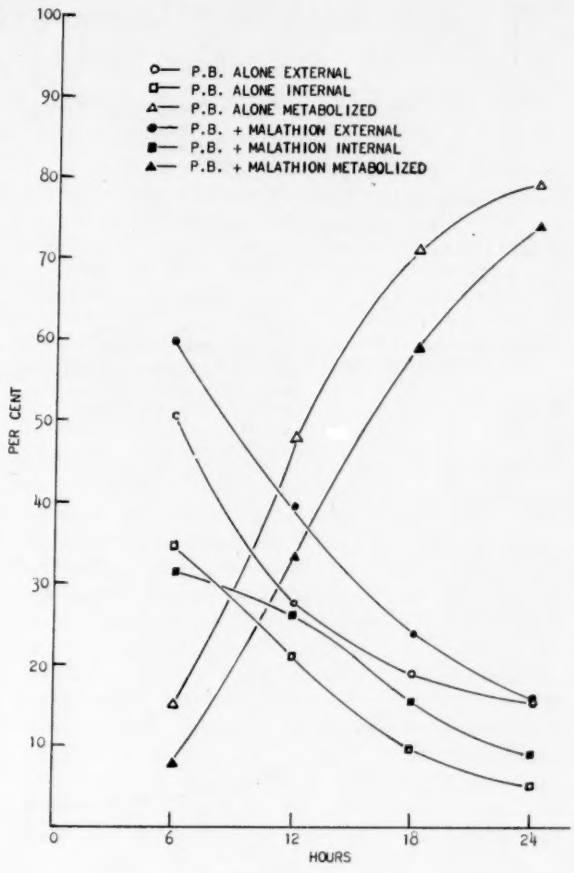

FIG. 1.—Recoveries of piperonyl butoxide alone and with malathion from topically-treated male house flies, and metabolism rates during antagonistic interaction.

when it is applied alone. However, at 24 hours the percentage of externally held piperonyl butoxide is relatively the same.

All internal extractions of the synergist alone indicate lower levels than when it accompanies either toxicant. Assuming that the synergist not recovered was metabolized (figures 1 and 2 illustrate this disappearance as metabolism), the trend of the points represents the rate of metabolism. The lower concentration of the compound in figure 2 shows a consistently higher percentage metabolized than the higher dosage of figure 1.

Penetration here is defined as the amount that was not recovered from external extractions. The penetration rates of piperonyl butoxide alone and with the toxicants are relatively the same. However, the rates are considerably different between the levels of 3.5 and 1.25 μg./μl. The indication here is that the higher concentration yields a lower percentage of penetration.

DISCUSSION.—According to Blum (1955) this color reaction is specific for compounds containing methylenedioxy or ethylenedioxy groups. Considering this fact and the decreasing level of synergist recovered internally with time, it may be stated that the end metabolites of

[3] Piperonyl butoxide, 100%, supplied by the Fairfield Chemical Division, Food Machinery and Chemical Corporation, Baltimore, Maryland.

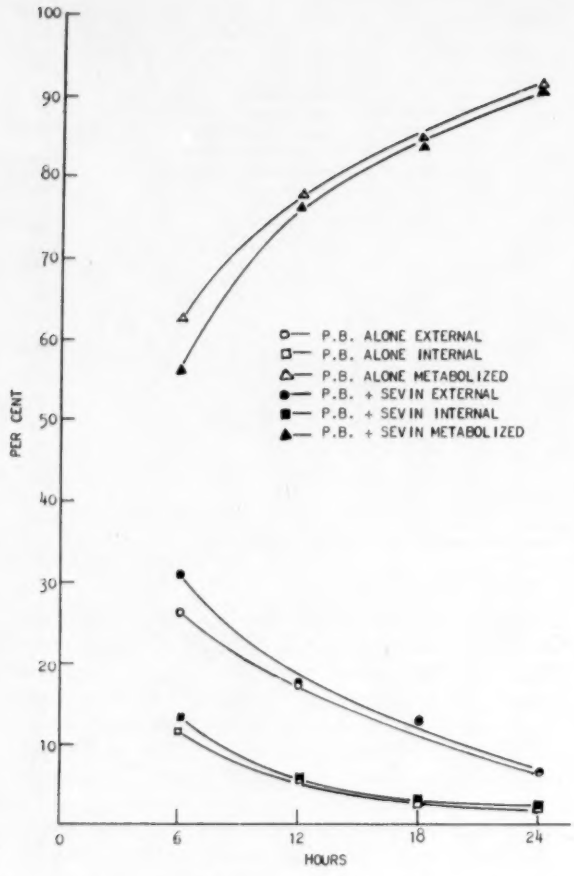

FIG. 2.—Recoveries of piperonyl butoxide alone and with Sevin from topically-treated male house flies, and metabolism rates during synergistic interaction.

piperonyl butoxide in the house fly contain neither of these groups.

Roan & Babers (1954), studying DDT penetration in the American cockroach, *Periplaneta americana* (L), stated that there appeared to be an optimum concentration per unit area beyond which there would be little or no effect on penetration over a relatively short period. This statement appears to be confirmed in this investigation, in that by percentage the smaller dosages of synergist penetrate faster, but quantitatively they are nearly the same.

Wilson (1949) stated that with separated doses of toxicant and synergist, external effects, such as increased penetration or protection of pyrethrum from oxidative destruction, could not play more than a small part in the synergistic action of piperonyl butoxide or piperonyl cyclonene.

Perry & Hoskins (1951), using piperonyl cyclonene as a synergist for DDT, stated that the effect of the synergist on DDT was not due to a change in the rate of DDT penetration. They observed in another study (1950) that DDT-resistant house flies converted more DDT to DDE than the nonresistant strain, and the addition of piperonyl cyclonene inhibited this conversion.

Reviewing the literature it appears that the commonly used synergists' action is not understood. Their antagonistic action is even less understood and investigated. Results of this work suggest that the synergistic or antagonistic interaction of piperonyl butoxide is not determined by the rate of its penetration with the toxicant in topical applications to male house flies.

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Effect of Methyl Bromide and Hydrocyanic Acid Fumigation on the Germination of Flax Seeds[1]

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Abstract

Two varieties of flax, Imperial and Punjab, with a graded moisture range of 6, 8, 10 and 12% were fumigated with methyl bromide in 100-cubic-foot gastight chambers at an exposure period of 72 hours. Various dosages and temperatures were used. One variety of flax seed, Imperial, was fumigated with methyl bromide and hydrocyanic acid under varying conditions of temperature, exposure and dosages of fumigant. One-half of the fumigated samples of seed received one fumigation; the others were fumigated twice. Two series of germination tests were made, the first within 5 days following fumigation of seeds, and the second after 84 days of post-fumigation storage at 50° F.

Under the conditions of the experiments, the germination of flax seeds was not affected by fumigation with hydrocyanic acid. It was apparent that methyl bromide fumigation may reduce the germination of flax seeds under some conditions, but the erratic nature of test results prevented isolation of variables according to their importance in contributing to germination injury.

Although seven species of insects have been recorded from flax seeds in California (Strong & Okumura 1958), ordinary measures for the control of storage pests seldom are considered necessary. However, heavy dosages of fumigant and long exposures are required to eradicate one species, the khapra beetle, *Trogoderma granarium* Everts, when infestations are found in seeds or storage facilities. The possibility of causing a reduction or failure of seed germination when fumigating to eradicate the khapra beetle was sufficient reason to evaluate several variables which may influence the germination of flax seeds exposed to methyl bromide or hydrocyanic acid. The purpose of this paper is to summarize results from germination tests with flax seeds which were exposed to these two fumigants under various controlled conditions.

Materials and Methods.—*Preparation of Seed Samples.*—Two varieties of flax seeds, Imperial and Punjab, were used in one series of tests (72 hours exposure period) with methyl bromide. Only one variety, Imperial, was used in tests throughout the study. The initial moisture content (m.c.) of Imperial flax was 5.3% and that of Punjab flax was 5.0%. Normal fluctuations in the moisture content of Imperial flax in storage ranged from 5.3% to 6.4% during the total experimental period of approximately 18 months.

Careful adjustment of moisture and conditioning of seeds resulted in the desired graded moisture range of 6%, 8%, 10% and 12%. Occasionally it was necessary to dry seeds, but most often water was added to increase the moisture to the desired level. After each moisture adjustment, seeds were conditioned in airtight polyethylene bags for 7 days at 50° F. Then seeds were blended and moisture tests were made to determine the need for further drying or addition of water. All moisture tests were made with a *Steinlite 400 G* moisture tester.

When the moisture content reached the desired level, each lot of seeds was divided into samples of approximately 30 grams. The samples were put into small rough-woven cotton bags and removed only for germination tests. Each cloth sample-bag of flax seeds was placed in an airtight polyethylene bag to prevent moisture changes while the prepared samples awaiting fumigation were held in storage at 50° F.

Fumigation of Seeds.—Test samples were removed from storage and held overnight at the desired fumigation temperature before removing airtight polyethylene bags. Seeds of all four moisture levels were placed in slatted crates inside the fumigation chambers which contained only the samples to be fumigated together. The crates permitted thorough circulation of gases.

Fumigation experiments were conducted in 100-cubic-foot gastight chambers with continuous circulation of air within the fumatorium. Temperatures were thermostatically controlled at 50°, 70° and 90° ± 1° F., but no attempt was made to control humidity. Fumigations were replicated twice.

Fumigants were applied at two dosage levels, one high and one low, in all tests. Dosages were selected according to exposure periods and temperatures during fumigation. When germination was impaired after exposing seeds to fumigants for 72 hours, tests were made with shorter exposure periods.

The high dosages of hydrocyanic acid used are given in table 1 for each of the three fumigation temperatures. Exposure periods, temperatures during fumigation, and dosages of methyl bromide applied are given in tables 2, 3, and 4. Concentrations of fumigants were not determined for each dosage or exposure interval, but typical curves for the fall in concentrations of methyl bromide were given previously by Strong & Lindgren (1959a).

All seeds received an initial fumigation, and one-half of the samples were fumigated a second time 21 days after the initial exposure to test the effect of repeat fumigation. After fumigation, seeds were aerated for 24 hours, then repackaged in airtight polyethylene bags. Samples were held in postfumigation storage at 50° F. and removed only for germination tests.

Germination Tests and Assessments of Results.—Two series of germination tests were made, the first within 5 days after fumigation of seeds, and the second after 84 days (12 weeks) of postfumigation storage at 50° F. Seeds were germinated between blotters at 68° F.

The percentages of germination (normal seedlings) were the only criteria recorded for comparison of results. (See p. 192, U. S. Dept. of Agric. Agricultural Handbook No. 30, 1952, for a description of normal and abnormal seedlings.) Unfumigated seeds served as control standards for comparison.

Results.—More than 2,000 germination tests of 100

[1] Paper No. 1171, University of California Citrus Experiment Station, Riverside, Calif. Accepted for publication July 14, 1959.

Table 1.—Corrected percentages of reduction in the germination of Imperial flax seeds after two fumigations with hydrocyanic acid for 72 hours at three temperatures and varying dosages.

Moisture Content Seeds (%)	Fumigation Temperatures and Dosages in Pounds per 1,000 Cubic Feet		
	90° F.—2.0	70° F.—2.5	50° F.—3.0
	Per cent Reduction in Germination		
6	1	0	0
8	1	0	2
10	0	0	0
12	0	3	4

Table 2.—Corrected percentages of reduction in the germination of Imperial flax seeds after one and two methyl bromide fumigations at 90° F. with various exposure periods and dosages.

Moisture Content Seeds (%)	Exposure Periods and Dosages in Pounds per 1,000 Cubic Feet									
	72 Hrs.		48 Hrs.		24 Hrs.		8 Hrs.		2 Hrs.	
	1.0	3.0	1.5	4.0	1.5	4.0	2.0	5.0	3.0	6.0
	Per Cent Reduction in Germination									
One fumigation										
6	7	22	8	21	0	3	0	2	0	0
8	7	15	0	7	0	0	0	0	3	8
10	6	17	3	9	3	6	2	5	1	3
12	9	11	1	6	2	11	4	7	4	0
Two fumigations										
6	14	13	13	30	8	22	4	8	0	1
8	0	14	7	20	7	23	11	8	0	0
10	8	24	7	30	0	15	7	8	0	7
12	0	0	13	21	12	23	10	8	18	11

Table 3.—Corrected percentages of reduction in the germination of Imperial flax seeds after one and two methyl bromide fumigations at 70° F. with various exposure periods and dosages.

Moisture Content Seeds (%)	Exposure Periods and Dosages in Pounds per 1,000 Cubic Feet									
	72 Hrs.		48 Hrs.		24 Hrs.		8 Hrs.		2 Hrs.	
	1.5	3.0	2.0	4.0	2.0	4.0	3.0	5.0	3.0	6.0
	Per Cent Reduction in Germination									
One fumigation										
6	0	0	12	31	13	12	1	0	9	11
8	0	0	5	16	6	8	7	3	2	2
10	10	19	14	20	10	11	10	14	0	0
12	10	19	17	16	15	21	6	9	19	8
Two fumigations										
6	11	32	25	33	25	20	9	4	7	0
8	3	20	13	22	2	12	5	7	0	0
10	10	12	9	26	11	24	0	12	2	0
12	11	18	10	22	6	22	4	12	5	10

seeds per test were made during the study, but only a condensed summary of data is presented. The results from fumigated seeds are given as the corrected percentages of reduction in germination as calculated by extending the use of Abbott's (1925) formula.

Differences in the percentages of germination in control samples of the two varieties of flax were negligible, and their response to methyl bromide fumigation for the 72-hour exposure period was quite similar. For this reason, only one variety of flax seed, Imperial, was used in all tests. A summary of data from germination tests with Imperial flax seeds is presented in this report.

Table 4.—Corrected percentages of reduction in the germination of Imperial flax seeds after one and two methyl bromide fumigations at 50° F. with various exposure periods and dosages.

Moisture Content Seeds (%)	Exposure Periods and Dosages in Pounds per 1,000 Cubic Feet									
	72 Hrs.		48 Hrs.		24 Hrs.		8 Hrs.		2 Hrs.	
	2.0	4.0	2.5	5.0	2.5	4.0	3.0	6.0	3.0	6.0
	Per Cent Reduction in Germination									
One fumigation										
6	1	4	4	13	1	1	0	0	2	6
8	4	6	3	8	5	9	1	0	6	2
10	4	10	16	14	4	12	1	1	6	0
12	3	18	13	26	13	22	5	0	0	1
Two fumigations										
6	4	10	15	27	13	14	0	0	11	2
8	1	12	18	25	5	7	0	1	0	11
10	12	23	29	31	11	19	8	7	2	1
12	18	30	22	45	9	9	4	3	6	7

Hydrocyanic Acid Fumigation.—Exposure of flax seeds with a graded moisture range of 6, 8, 10 and 12% to hydrocyanic acid fumigation for 72 hours at temperatures and dosages listed in table 1 did not result in significant reduction in germination. It is apparent from these data that further discussion is unnecessary.

Methyl Bromide Fumigation.—Results from the first series of germination tests (seeds planted within 5 days after fumigation) are summarized in tables 2, 3, and 4 as the corrected percentages of reduction in germination. From the data presented, it is obvious that under some conditions the germination of flax seeds was impaired by fumigation with methyl bromide. The results, however, are noticeably erratic and it is not possible to differentiate the cause of effects as easily as in previous reports concerning seeds of cereal grains (Strong & Lindgren 1959, a, b, c, d).

Two of the seven variables considered, varietal differences and postfumigation storage of seeds, obviously were of no importance and data concerning these two variables are not presented. All other variables—moisture content of seeds, period of exposure to fumigant, temperature during fumigation, dosage of fumigant, and repeat fumigation—probably were of some importance in contributing to injury of seeds during fumigation with methyl bromide. However, the erratic nature of the results in tables 2, 3, and 4 does not favor a prolonged discussion. It is apparent that methyl bromide fumigation may reduce the germination of flax seeds under some conditions and should be used with discretion for the fumigation of seeds to be used for planting.

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Heptachlor Residues on Corn Stover in Relation to Dairy Cattle Feeding[1]

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ABSTRACT

A field of dent corn was treated with a granulated formulation containing 5% of heptachlor, at the recommended time and dosage (1 pound heptachlor per acre), for control of first-brood European corn borers, *Pyrausta nubilalis* (Hbn.). Corn samples were analyzed for heptachlor and heptachlor epoxide at intervals after insecticide application. Holstein dairy cows were pastured in this field after ear harvest and milk collected from them at regular intervals. Butter was made from this milk. Extracts of butterfat were analyzed colorimetrically for heptachlor epoxide. Because there was no significant difference between the absorbance values of control and test samples, it was concluded that no heptachlor epoxide was secreted in the milk of the test cows.

This experiment is an outgrowth of previous experiments conducted at the European Corn Borer Laboratory at Ankeny, Iowa in which Cox *et al.* (1956) reported that heptachlor was effective, in granulated formulations, in controlling the European corn borer, *Pyrausta nubilalis* (Hbn.).

Davidow & Radomski (1953) isolated heptachlor expoxide from the adipose tissues of heptachlor-fed dogs. Radomski & Davidow (1953) found that heptachlor epoxide accumulated primarily in the fatty tissues in both rats and dogs fed heptachlor. Davidow *et al.* (1953) detected the same metabolite in the milk of a dairy cow fed heptachlor. Ely *et al.* (1955) could find no heptachlor epoxide in the milk of cows fed less than 500 mg. of heptachlor per cow per day. This epoxidation of heptachlor also occurs in the soil (Gannon & Bigger 1958) and on plants (Gannon & Decker 1958).

Farmers in the Corn Belt frequently pasture their cattle in corn fields after ear harvest. The cattle pick up ears missed in harvesting and also feed on the stover. The objective of this experiment was to determine if any heptachlor epoxide would be secreted in the milk of dairy cattle allowed to pasture on corn stover remaining in the field after harvest of heptachlor-treated corn.

MATERIALS AND METHODS.—*Insecticide Application and Corn Sampling.*—Two fields of late-planted dent corn at the Iowa State University farm near Ankeny, Iowa were used. One field (4.7 acres) served as an untreated control and a second (4.4 acres) was treated with a granulated formulation containing 5% of heptachlor at the rate of 20 pounds per acre (1 pound actual heptachlor per acre). The heptachlor formulation was prepared using 30/60 mesh Attaclay and a heavy aromatic solvent at the rate of 0.013 of a gallon per pound of finished formulation. This treatment was applied on July 11, 1957, when the corn was in the late-whorl stage. A fluted-feed, granular applicator mounted on a high-clearance vehicle was used to apply the insecticide (Lovely *et al.* 1956).

Samples of corn from both fields were collected 1 day, and 4, 8, 18, 32, and 102 days after insecticide application. The corn was picked during the first week in November and samples of the stover were collected on November 8, 120 days after insecticide application and 13 days prior to pasturing the dairy cows on the stover. Four composite samples were obtained each sampling day. A composite sample consisted of 10 plants selected at random from each plot. Each composite sample was chopped, mixed thoroughly, and a 1-quart aliquot selected for residue analysis. These samples were shipped to Vincennes, Indiana for analysis.

Analysis of Corn Samples.—The samples of chopped and minced corn plants were transferred to glass jars, a suitable quantity (usually 300 to 700 ml.) of colorimetric grade, normal pentane added and the samples tumbled in an end-over-end rotator for 2 hours. The solvent was then poured off into sample bottles. The analytical procedures used for the corn and stover samples were based on the methods of Ordas *et al.* (1956).

For the analysis of heptachlor residues, appropriate aliquots of extracts from samples with residues over 0.25 p.p.m., were clarified in test tubes by shaking with 2 gm. of Florisil[5] (a magnesia-silica gel adsorbent). The clarified extracts were filtered into test tubes, the Florisil washed twice with small amounts of pentane, and almost all of the solvent removed in a 50° C. water bath. The remainder of the solvent was removed with a gentle stream of dried air. The residue was dissolved in 1 ml. of benzene and 1 ml. of Davidow reagent (Davidow 1950) added. The tubes were placed in a hot oil bath (100 ± 1° C.) for 30 minutes, cooled, and their contents diluted to 5 ml. with methanol. The absorbance of the solutions was read in a Beckman, model B, spectrophotometer at a wave length of 567 mμ. in 10 mm. square cuvettes. Recoveries of known amounts of heptachlor averaged about 94% using these techniques.

Appropriate aliquots of extracts from samples with residues below 0.25 p.p.m. were concentrated to a volume

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[5] 60/100 mesh, Floridin Co., Tallahassee, Fla.

of 10 ml. and chromatographed through a 10-gm. column of activated Florex[6] contained in a 50-ml. Mohr buret and wetted with pentane. The Florex was activated by washing with pentane, removing the solvent under partial vacuum, and heating at 140° C. for 24 hours. The column was eluted with 200 ml. of pentane. The eluant was collected in a 250-ml. Erlenmeyer flask, a 3-ball Snyder distilling column was attached to the flask, and the eluant was reduced to a volume of about 5 ml. in a 50° C. water bath. The concentrated eluant was transferred quantitatively to a micro-reaction tube and the remaining pentane evaporated in a 40° C. water bath, using a gentle stream of dried air to facilitate evaporation. To each tube was added 0.2 ml. of modified Polen-Silverman reagent and the tubes placed in a 100° C. oil bath for 15 minutes. The tubes were cooled and their contents diluted to 0.5 ml. with a benzene: isopropanol solution (4:1, volume/volume). The solutions were transferred to micro-cuvettes and the absorbencies of the solutions read in the spectrophotometer at a wave length of 567 mμ. Recoveries of known amounts of heptachlor averaged about 88% using these procedures.

For the analysis of heptachlor epoxide residues, appropriate aliquots of the pentane extracts were concentrated to about 10 ml. on the 50° C. water bath using 250-ml. Erlenmeyer flasks and 3-ball Snyder columns. These solutions were transferred quantitatively to chromatographic columns containing 10 gm. of activated Florex wetted with pentane. The column was eluted with 200 ml. of pentane and the eluant discarded. The column was then eluted with 200 ml. of ether-pentane solution (6% diethyl ether[7] in pentane, volume/volume) and the eluant collected in a 250-ml. Erlenmeyer flask. A 3-ball Snyder column was attached to the flask and the eluant concentrated to about 5 ml. in a 50° C. water bath. The residue was transferred quantitatively to micro-reaction tubes and evaporated to dryness in a 40° C. water bath. The tubes, after adding 0.2 ml. of modified Polen-Silverman reagent, were placed in a 100° C. oil bath for 15 minutes, cooled, and their contents diluted to 0.5 ml. with the benzene:isopropanol diluent. The solutions were transferred to micro-cuvettes and the absorbencies of the solutions were read at a wave length of 410 mμ. Recoveries of known amounts of heptachlor epoxide averaged about 90% using these procedures.

Pasturing of Dairy Cows and Milk Processing.—On November 21, 133 days after insecticide application, two healthy Holstein cows, in the sixth or seventh month of lactation, were randomly assigned to each of the control and heptachlor-treated fields. Each field was enclosed with electrified wire. The cows were pastured on the fields for 2, 4, and 6 hours on the first, second, and third days, respectively, and 7.5 hours per day thereafter. They were pastured on the corn stover, during daylight hours, for 38 consecutive days. The remainder of the time they were kept in a conventional loose-housing barn and were offered good quality, alfalfa hay free choice. The cows received 10 pounds of grain daily prior to and during the experimental period.

Composite milk samples from the evening and following morning milkings of each group of cows were collected twice-weekly. Daily milk weights for each cow were recorded. The milk was heated to about 35° C. and the cream was separated mechanically and stored overnight at 4° C. The chilled cream, at a temperature of about 10° C., was poured into a motor-driven Dazey churn. The cream was churned until butter formed. The buttermilk was poured off and the butter washed with cold, distilled water until the washings were clear. The butter was placed in polyethylene bags and stored at −15° C.

Samples of the whole milk, skim milk, cream, and buttermilk were taken to obtain estimates of fat percentages (Herreid 1948) and as a check on processing procedures. Portions of the butter samples were analyzed for per cent fat, moisture, and curd.

Analysis of Butterfat for Heptachlor Epoxide.—The butter samples were analyzed in the Department of Zoology and Entomology, Iowa State University at Ames. The frozen butter samples were softened at room temperature and about 150 gm. of each placed in a large mortar. Anhydrous sodium sulfate was added to the sample in an approximate ratio of 4:1. The butter and drying agent were ground with a pestle until a homogeneous, friable mixture was obtained. The mixture was transferred to a glass jar and stored at −15° C. After being stored for at least 24 hours, portions of the mixture were packed in an extraction thimble which was placed in a Soxhlet apparatus and extracted with colorimetric grade, normal pentane for 2 hours. Successive portions of the mixture were extracted in this manner until the entire sample was processed. The pentane solution of fat was centrifuged to remove solid materials carried over during extraction. The clear, supernatant solution was transferred to an Erlenmeyer flask and most of the pentane removed on a 50° C. water bath using a 3-ball Snyder column. The flask was removed from the bath and the small amount of pentane remaining was removed by using a gentle current of dried air. The pure butterfat was stored in glass bottles at −15° C. until needed for analysis.

The quantitative estimation of heptachlor epoxide in the butterfat was based on methods developed by Meyer *et al.* (1958). The butterfat sample was melted on a water bath and 10 gm. of fat transferred to a 250-ml. Erlenmeyer flask. Fifteen milliliters of 95% ethanol and 10 ml. of 50% aqueous KOH (weight/volume) were added to the flask. The flask was fitted with a water-cooled condenser and the contents refluxed in boiling water for 5 minutes. The inner tube of the condenser was rinsed with about 15 ml. of distilled water, the condenser was detached, and the flask and contents cooled to room temperature by immersion in cold water. The contents of the flask were transferred quantitatively to a 500-ml., Squibb-type, separatory funnel. The saponification flask was rinsed twice with two 10-ml. portions of pentane and the rinsings added to the funnel. To the funnel were added 190 ml. of pentane. The funnel and contents were gently rocked to and fro for about 1 minute. Hard shaking was avoided to prevent the formation of emulsions. After the layers had separated, the lower, aqueous phase was drawn off into a second, separatory funnel and re-extracted with an additional 100 ml. of pentane. After the layers had separated, the lower, aqueous phase was discarded. The

[6] XXS grade, 60/100 mesh, Floridin Co., Tallahassee, Fla.
[7] Ethyl ether, spectro grade, cat. no. S 997, Eastman Organic Chemicals, Distillation Products Industries, Rochester N. Y

two pentane extracts were washed separately; first with 100 ml. of acidified, distilled water (2 ml. of 1 *N* HCl per 100 ml. of distilled water) and second with 100 ml. of distilled water. The water washes were discarded and the pentane extracts combined in the first funnel. The second funnel was rinsed twice with 10-ml. portions of pentane and the rinsings added to the first funnel. The combined extracts were dried by allowing them to filter through an approximately 3-cm. layer of anhydrous sodium sulfate contained in a cylindrical funnel plugged with glass wool. The separatory funnel was rinsed twice with 10-ml. portions of pentane and the rinsings passed through the sodium sulfate. The sodium sulfate was also rinsed with pentane. The dried, pentane extract was concentrated to about 10 ml. on a 50° C. water bath using a 3-ball Snyder column attached to a 500-ml. Erlenmeyer flask.

Column chromatography was used to isolate the heptachlor epoxide from other saponification products in the pentane extract. The chromatographic column consisted of a glass tube, 20 mm. in outside diameter by 250 mm. long, fitted with a glass stopcock and an expanded top which provided a reservoir with a capacity of about 250 ml. The column was prepared by inserting a small glass-wool plug at the bottom of the tube and pouring in 10 gm. of pentane washed Florex that had been activated as described above. The adsorbent was poured into the tube in three portions and each portion gently tamped to pack the column.

A 500-ml. Erlenmeyer flask was attached to the chromatographic column and about 20 ml. of pentane poured onto the column, with the stopcock open, to wet the adsorbent. The stopcock was closed and the concentrated, yellow, pentane extract quantitatively added to the column. The stopcock was opened and, when most of the extract and washings had passed into the column, the tube portion of the column was washed down with 10 ml. of pentane. The column was eluted with 180 ml. of pentane and the stopcock closed when the eluant level reached the top of the adsorbent. The flask was detached and the pentane eluate discarded. A clean flask was attached to the column, the stopcock opened, and the column eluted with 200 ml. of ether-pentane solution (2% diethyl ether in pentane, volume/volume). The stopcock was closed as the eluant reached the top of the adsorbent. The flask was detached and the ether-pentane eluate concentrated to about 10 ml. of a 50° C. water bath using a 3-ball Snyder column. The flask was removed from the bath and the colorless concentrate further evaporated to a volume of about 2 ml. with a gentle stream of dried air. The concentrated extract was quantitatively added to a micro-reaction tube and the pentane evaporated on a water bath at 40° C.

One milliliter of modified Polen-Silverman reagent was added to each micro-reaction tube. The tube was heated for 15 minutes in an oil bath (temperature 100±1° C.) and cooled to room temperature by immersion in cold water. The yellow-colored solution was diluted to 2.5 ml. with the benzene:isopropanol diluent. After complete mixing, this solution was transferred to a 10-mm., square cuvette and its absorbance read at a wave length of 415 mμ. with a slit width of 0.035 mm., using a Beckman, model DU, spectrophotometer. A pentane solution containing 10 μg. heptachlor epoxide[8] per ml. was used to prepare a standard calibration curve each time samples were analyzed. Usually 5, 10, and 20 μg. of heptachlor epoxide were used to prepare this curve. Preparation of spectral absorbence curves confirmed that maximum light absorption occurred at 415 mμ. (Meyer *et al.* 1958). Additional heptachlor epoxide was dissolved in control, pentane-extracted butterfat to give a concentration of 5 μg. per gm. of fat. Duplicate mixtures, consisting of 2-gm. portions of this butterfat solution of heptachlor epoxide plus 8 gm. of control butterfat, were analyzed with each set of test samples to estimate the efficiency of the analytical procedure. From each set of milk samples, six butterfat samples were analyzed as follows: 2 control samples (milk from cows on the untreated field), 2 test samples (milk from cows on the heptachlor-treated field), and 2 recovery samples prepared as described in the preceding sentence.

RESULTS AND DISCUSSION.—*Residues on the Corn and Stover Samples.*—The mean heptachlor residues found on the corn samples collected 1 day and 4 days after insecticide application were 1.44 and 0.55 p.p.m., respectively. The samples collected 8, 18, 32, and 102 days after insecticide application all contained heptachlor residues of less than 0.01 p.p.m. These values are based on the harvest weight of the samples; the per cent moisture in the 6 sets of samples was estimated to be as follows: 85.4, 88.1, 83.1, 79.3, 78.5, and 49.0. The samples collected 1 day, and 4, 8, 18, and 32 days after insecticide application were also analyzed for heptachlor epoxide. The mean epoxide residues in these samples were 0.04, 0.15, 0.07, 0.06, and 0.03 p.p.m., respectively. No heptachlor residues could be detected in the stover samples collected 120 days after insecticide application.

Butterfat Analyses.—Eleven composite milk samples were collected from both groups of cows during the 38 days they were pastured on the two fields. The corrected absorbence values of the 22 butterfat samples analyzed for heptachlor epoxide fluctuated closely about zero, using a reagent blank to establish a zero setting on the spectrophotometer. The mean absorbence value and standard error of the mean for the test (epoxide) samples was 0.011±0.001, the corresponding value for the control samples was 0.008±0.001. The 95% confidence interval of the absorbence values for the test and control butterfat samples was 0.011±0.003 and 0.008±0.002, respectively. The 99% confidence interval of the absorbence values for the same samples was 0.011±0.004 and 0.008±0.003, respectively. The overlapping of the confidence intervals suggests that there was no significant difference between the absorbence values of test and control butterfat samples. Therefore, there is no evidence, at the specified probability levels, that heptachlor epoxide was present in the butter samples. The mean recovery of 10 μg. quantities of heptachlor epoxide carried through the analytical procedures was about 82% (range 73% to 90%).

The procedures used to analyze the butter fat samples provided a means of detecting accurately about 1 μg. of heptachlor epoxide in a 10-gm. sample of fat. This was equivalent to being able to estimate 0.1 p.p.m. of heptachlor epoxide in a 10-gm. fat sample or about 0.003

[8] Analytical grade (99.5%) obtained from the Velsicol Chemical Corporation, Chicago, Ill.

p.p.m. of the epoxide in a 300-gm. sample of whole milk (assuming about 3.5% butterfat).

Dairy Cow Production Records.—The number of cows in each experimental group was necessarily small and therefore, it was not possible to draw conclusions about the effect of heptachlor-treated forage on milk and butterfat production. The mean daily milk weight and the standard error of the mean for the control and test cows were 52.7 ± 0.6 and 50.1 ± 0.6 pounds, respectively. The mean butterfat values of the milk from the two groups of cows were 3.6% and 3.4%, respectively.

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A Comparison of the Toxicity of Insecticides for the Control of Corn Earworm on Sweet Corn[1]

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ABSTRACT

Experiments to control the corn earworm, *Heliothis zea* (Boddie), have been conducted in southern California with 69 different formulations during the past 10 years. These individual-ear, paint-brush dust tests showed Sevin® (1-naphthyl-*N*-methyl carbamate), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), heptachlor, Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), and isodrin equal to or slightly better than a 5% DDT standard treatment. The higher dosages of malathion, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) and Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) approached DDT in effectiveness, while all other materials were poor or ineffective.

Between 1948 and 1958 many experiments have been conducted for the control of corn earworms, *Heliothis zea* (Boddie), on sweet corn in southern California. In these studies attention has been given to method, time, and number of treatments, as well as to insecticide formulations, in an attempt to determine the best control recommendations. The results of some of these tests between 1948 and 1952 have been reported by Anderson & Hashe (1949), Anderson *et al.* (1951a, 1952) and Bacon *et al.* (1953). The following is a summary of the effectiveness of 69 formulations that have been tested during the past 10 years.

METHODS.—In all these tests, treatments were made by the individual-ear paint-brush dust method (fig. 1). For this treatment method, a 1- to 1.5-inch stencil-type paint brush was used. The dust was carried in a wide-mouthed container strapped around the operator's waist. The brush was pushed into the dust and then transferred with a slight but sudden thrust into the silk of the ear. With practice, two to three ears may be treated satisfactorily with one dipping of the brush into the dust. In these experiments, treatments were started within a day after the first few ears began silking, and repeated applications were made at 3-day intervals for either three or four times (the number of treatment times varied with the season and the year). In most instances plots were one row wide and 50 to 100 feet long and were replicated four times for each test material. Twenty-five ears were examined for earworm injury in each replicate (total 100 ears) for each treatment. Ears were recorded as clean or injured.

The population of the corn earworm is probably the most important single factor influencing the insecticidal control of this pest on sweet corn in southern California (Anderson *et al.* 1951b). Since these tests were made under

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Table 1.—Comparative toxicity of 69 formulations tested against corn earworms on sweet corn in southern California during 1949 to 1958, inclusive.

Insecticide Dust[a]	(%)	No. Years Tested	No. Tests	Ears Clean/100 (% Control): Test Material	5% DDT Standard	Check	Per Cent Difference Between Treatment and Standard
Sevin®	10	1	1	92	85	0	+ 7
	5	2	4	93	92	0	+ 1
	2	1	1	91	90	0	+ 1
Thiodan®	2	3	4	90	88	0	+ 2
	1	1	1	83	90	0	− 7
Heptachlor	3	2	2	88	86	32	+ 2
	2.5	2	2	82	90	0	− 8
Endrin	2	1	1	99	98	0	+ 1
	1	1	1	94	94	63	0
Dipterex®	10	2	3	97	96	11	+ 1
	5	4	8	92	91	4	+ 1
Isodrin	1	1	3	92	94	5	− 2
C-25141	2	1	1	87	90	0	− 3
Malathion	5	1	1	73	76	0	− 3
	4	1	3	68	85	0	−17
	2	1	1	68	85	1	−17
Malathion[b]	4	1	2	53	80	0	−27
Diazinon®	4	4	4	85	90	0	− 5
	2	1	1	91	98	0	− 7
Guthion®	5	1	1	75	82	0	− 7
	2	1	1	55	76	0	−21
SD-4402	2	1	1	82	90	0	− 8
C-8305	10	1	1	88	96	0	− 8
	5	1	1	81	96	0	−15
	1	1	1	62	96	0	−36
Parathion	1	1	1	77	88	3	−11
Kepone®	10	1	1	86	97	5	−11
	5	1	1	81	97	5	−16
TDE	5	2	2	74	86	2	−12
	3	1	1	71	83	3	−12
DDT	2	1	1	63	76	0	−13
	1	2	2	73	86	3	−13
	0.5	1	1	32	76	0	−43
Dilan	3	1	1	60	73	2	−13
	1	1	1	84	94	5	−10
Ethion	4	1	1	84	97	0	−13
Perthane®	10	2	2	70	84	2	−14
	5	3	3	57	81	2	−24
	2	1	1	11	76	0	−65
	1	1	1	10	76	0	−66
Ronnel	2	1	1	66	88	0	−22
C-6812	5	1	1	67	89	0	−22
C-4018	2	1	1	63	85	1	−22
C-3975	2	1	1	63	85	1	−22
Chlorthion®	4	1	1	74	97	0	−23
	2	1	1	28	85	1	−58
Ryania	40	1	1	62	88	3	−26
	100	1	1	31	91	0	−60
Trithion®	5	2	4	62	88	0	−26
Q-128	5	1	1	40	73	2	−33
Methoxychlor	10	1	1	36	76	0	−40
	5	2	2	48	81	3	−33
Dibrom®	4	1	1	38	83	0	−45
	2	1	1	20	83	0	−63
Soapstone	100	1	1	50	97	5	−47
Toxaphene	5	1	1	26	76	0	−50
Dicapthon	2	1	1	47	97	0	−50
Lindane	1	1	1	36	88	3	−52
Dieldrin	0.5	1	1	34	88	3	−54
Dow M-350	2	1	1	22	81	0	−59
Delnav®	2	1	1	29	88	0	−58
Pyrethrins	0.23	1	1	2	73	2	−71
Phostex®	5	1	1	5	82	0	−77
MGK R-11	5	1	1	2	83	0	−81
MGK R-326	5	1	1	0	83	0	−83
Bacillus[c]		1	6	2	87	0	−85
Aldrin	1	1	1	3	88	3	−85
Tabutrex	5	1	1	2	96	0	−94
Pyrolite	100	1	1	0	96	0	−96

[a] Chemical definitions of all materials are given either in "Common Names of Insecticides" in Jour. Econ. Ent. **52**(2): 361–2, 1959, or in the following:

Union Carbide Chemicals Co.
Sevin = 1-naphthyl *N*-methyl carbamate.
UCC 6812 = 9,9′-difluorylidene.
UCC 8305 = (*P*-chloro-2,4-dioxa-5-methyl-*P*-thiono-3-phosphabicyclo (4.4.0) decane).

Niagara Chemical Division, Food Machinery & Chemical Corporation.
Thiodan = 6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide.
Phostex = bis(dialkyloxyphosphinothioyl) disulfides (alkyl ratio 25% isopropyl, 75% ethyl).

Chemagro Corporation, and Farbenfabriken Bayer.
Dipterex = *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate.
Guthion = *O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl)-phosphorodithioate.
Chlorthion = *O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate.
Compound 25141 = *O,O*-diethyl *O*-*p*-methylsulfinylphenyl phosphorothioate.

Geigy Chemical Corporation.
Diazinon = *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate.

General Chemical Co.
Kepone = 1,2,3,5,6,7,8,9,10,10-decachlorotetracyclo [5.2.1.0^{2,6}.0^{3,9}.0^{5,8}] decan-4-one.

The Dow Chemical Co.
Compound M-350 (ET-15) = *O*-methyl *O*-(2,4,5-trichlorophenyl)phosphoramidothioate.

California Spray-Chemical Corporation.
Dibrom = *O,O*-dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl) phosphate.

Rohm & Haas Co.
Perthane = 1,1-dichloro-2,2-bis(*p*-ethylphenyl) ethane.
Compound *O*-128 = 1,1,1-trichloro-2,2-bis(ethylphenyl) ethane.

Hercules Powder Co.
Delnav = 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate).

Stauffer Chemical Co.
Trithion = *S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate.

Commercial Solvents Co.
Dilan = 1 part 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts 1,1-bis (*p*-chlorophenyl)-2-nitrobutane.

Glenn Chemical Co.
Tabutrex = di-*n*-butyl succinate.

McLaughlin-Gormley-King Co.
R-326 = di-*n*-propyl isocinchomeronate.
R-11 = 2,3,4,5-bis(Δ^2-butylene) tetrahydrofurfural.

American Cyanamid Co.
Compounds 4018 (*O,O*-diethyl *S*-(1,2-dicarbomethoxyethyl) phosphorodithioate) and 3975 (*O,O*-diethyl *S*-(1,2-dicarboethoxyethyl) phosphorodithioate.

Shell Chemical Corporation.
Compound SD 4402 = 1,3,4,5,6,7,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan.

[b] This treatment was with a specially refined lot of malathion.

[c] *Bacillus thuringiensis* was tested at 10, 3.3 and 1.65 billion spores per gram dosages and all resulted in the same control; therefore, only one set of figures is given here.

FIG. 1.—Brush-dust method of individual ear treatment.

varying conditions over a long period, a 5% DDT material was included for comparison as a standard treatment in all tests. In addition, untreated check plots were included. Although nearly 100% of the corn ears were wormy in most of the tests, it should be noted that nearly 100% infestation may have resulted from single or multiple populations per ear. In view of these facts, the best measure of the effectiveness of a given material can be obtained by comparing it with the results obtained with the DDT standard treatment used in the same test.

In evaluating the effectiveness of these various materials it should be remembered that the application made in these individual-ear-treatment tests may be considered thorough and heavy (30 to 40 pounds of dust per acre per application). Therefore, control of earworms may be poorer with the lower dosages and less thorough coverage normally used on other crops. This fact was borne out in a few tests on sweet corn where it was found that some of the materials that gave good control as an individual ear treatment failed when applied by less thorough fixed-boom dust or spray methods.

DISCUSSION OF RESULTS.—The results of these tests, as given in table 1 (see footnote a to table 1 for chemical definitions), show that Sevin, Thiodan, heptachlor, endrin, Dipterex and possibly isodrin were equal to or slightly better than the 5% DDT treatment. One of the experimental phosphates and the higher dosages of malathion and Diazinon were about equal to the 5% DDT. Compound SD-4402, and the higher dosages of Guthion and Compound 8305, approached DDT in effectiveness. All other materials ranged from poor to no earworm control.

Taking into consideration earworm control and the residue problem, these data indicate that Sevin and Dipterex may have a place in future recommendations for treating sweet corn.

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Relationship between Metabolism and Differential Toxicity in Insects and Mice of Diazinon, Dimethoate, Parathion, and Acethion[1]

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ABSTRACT

The persistence and metabolism of Diazinon®, (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) dimethoate, parathion, and acethion (*O,O*-diethyl *S*-carboethoxymethyl phosphorodithioate) have been studied in the mouse, American cockroach (*Periplaneta americana* (L.)), and house fly (*Musca domestica* L.). The results have been used to explain the selective toxicity of three of these compounds toward insects as compared with mammals. For Diazinon, selectivity is attributed to high levels of oxygen analog in the susceptible species. For dimethoate and acethion, selectivity is attributed to a persistence of unaltered parent compound in the whole body. Small differences were found in Diazinon absorption and metabolism by normal and Diazinon-resistant house flies.

The physiological basis for the selective toxicity of organophosphate insecticides between insect species, and even more important between insects and mammals, deserves considerable attention. Metabolic relationships similar to those found for species selectivity may also account for the increasingly important phenomenon of organophosphate resistance.

In every case that has been studied, toxic phosphorothionates are converted in part to their phosphate analogs in living organisms. It is these oxygen analogs which are probably the actual toxicants, since they are almost always more potent anticholinesterases than their parent compounds. The level of phosphate analog produced in the body might be expected to be associated with toxicity. Thus in selective insecticides, one would expect high levels of phosphate analog in susceptible species only. In the case of malathion (Krueger & O'Brien 1959) this premise appeared to be true with respect to the susceptible house fly (*Musca domestica* L.) and cockroach (*Periplaneta americana* (L.)) as compared with the mouse.

The present study was designed to examine the importance of metabolism in determining the toxicity to insects and mammals of several insecticides of varying selectivity. The possibility that metabolic differences could account for the resistance of certain house fly strains to organophosphates has also been examined.

METHODS.—*Syntheses.*—Radioactive dimethoate was prepared, purified, and characterized as described previously (Dauterman *et al.* 1959) using isotopic exchange to obtain the phosphorus-32 pentasulfide intermediate (Casida 1958). The compound was formed in 60% yield with an approximate specific activity of 50 mc. per gram.

Radioactive acethion (*O,O*-diethyl *S*-carboethoxymethyl phosphorodithioate) (O'Brien *et al.* 1958) was prepared using the isotopic exchange method (Casida 1958) to obtain the phosphorus-32 pentasulfide intermediate. Potassium *O,O*-diethyl phosphorodithioate was was prepared in the same manner as previously described for dimethoate except that absolute ethanol was substituted for absolute methanol. The aqueous solution of potassium *O,O*-diethyl phosphorodithioate was diluted with twice the volume of acetone and added dropwise with stirring and refluxing to an equimolar amount of ethyl chloroacetate. The mixture was refluxed with stirring for 18 hours. Twice the volume of water was added to the reacted solution, and part of the acetone was removed by evaporation. The aqueous solution was then extracted three times with equal volumes of chloroform, which was then dried over anhydrous sodium sulfate and evaporated to dryness. The compound was purified by partition chromatography on a celite-iso-octane-methanol column (Bowman & Casida 1957) and obtained in 53% yield with a specific activity of about 50 mc. per gram.

Radioactive Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) was prepared using isotopic exchange to obtain labeled phosphorus pentasulfide, which was then converted to the phosphorus-32 trichloride intermediate (Casida 1958). *O,O*-diethyl phosphorochloridothionate was prepared according to Fletcher *et al.* (1948). The benzene solution was filtered, washed with water, dried over sodium sulfate, and added dropwise to a flask containing equimolar amounts of potassium carbonate and 2-isopropyl-4-methyl-6-hydroxypyrimidine in refluxing benzene. The contents of the flask were refluxed with stirring for 12 hours. The benzene solution was washed with equal volumes of water, 10% sodium hydroxide, 10% hydrochloric acid and finally with water. The product was purified as with acethion. Diazinon was obtained in 40% yield with a specific activity of about 35 mc. per gram.

Radioactive parathion was prepared from the thiophosphoryl-32 trichloride intermediate which was obtained by isotopic exchange (Vigne & Tabau 1958). *O,O*-diethyl phosphorochloridothionate was prepared as described above and was added dropwise to refluxing acetone containing an equimolar amount of the sodium salt of *p*-nitrophenol. The mixture was refluxed with stirring for 5 hours. The acetone solution was filtered to remove the NaCl and then evaporated and the residue dissolved in benzene. The benzene solution was washed with 10% sodium carbonate and then with water and evaporated. Parathion was purified as described for acethion and the product was obtained in 42% yield with a specific activity of about 25 mc. per gram.

Treatments and Extraction.—Topical applications were used for some toxicity determinations, and for study of the metabolism of normal and resistant house flies. The application techniques were identical with those described in a previous paper (Krueger & Casida 1957). Injections were used in all other cases, and for insects these were made with a micrometer-driven syringe fitted with a no. 30 hypodermic needle. The American cockroach was in-

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jected with 10 μl. at the femoro-tibial joint. The house fly was injected with 0.4 μl. in the thorax. After the dose was delivered, the insect was left on the needle for about 10 seconds to relieve back pressure and consequent loss of injected dose by bleeding. Mice were injected intraperitoneally with 0.1 ml. per 20 gm. body weight. For all animals, the solvent used was propylene glycol in the case of acethion, Diazinon and parathion, and isotonic saline in the case of dimethoate.

The extraction and separation techniques for acethion, Diazinon, and parathion were similar to those previously described for malathion (Krueger & O'Brien 1959). As before, about 10% to 20% of the radioactivity could not be extracted from the animal proteins. However with these three compounds, during the chromatographic separation on alumina only a small percentage (5% to 10%) of the added chloroform extractibles were not eluted with the solvents used. The portion remaining on the column was not identified or taken into account in calculating the level of P=O analog. Dimethoate and its P=O analog were quantitatively separated by a silica gel chromatographic system (Dauterman *et al.* 1959). The results reported are averages from three replicates of one mouse or 4 grams of insects per replicate.

Animals.—The resistant strain of house flies was kindly supplied by Dr. A. J. Forgash (Rutgers University) and is designated Rutger's "A" strain, selected for Diazinon. The resistant flies were reared through one generation in our laboratory without Diazinon pressure before use. Flies taken from the next generation showed a 40-fold resistance to Diazinon, in agreement with a previous report for this strain (Forgash 1959) and were used in the metabolism studies.

The susceptible strain was the Chemical Specialties Manufacturers Association (C.S.M.A.) 1948 strain obtained from the Wisconsin Alumni Research Foundation, Madison. Four-day-old female house flies were used throughout this investigation for both LD_{50} determinations and metabolism studies. American cockroaches were raised from a stock kindly provided by Dr. E. E. Kenaga, Dow Chemical Co. German cockroaches (*Blattella germanica* (L.)) were raised at the Wisconsin Alumni Research Foundation. Only male American and German cockroaches were used for LD_{50} and metabolism studies. Mice were white 15- to 25-gram females, from Rolfsmeyer Mouse Farms, Madison.

RESULTS.—In the following description, the term "chloroform extractibles" includes parent compound along with P=O analog. "Chloroform inextractibles" (labeled as "H_2O" fraction in the figures) includes the ionic degradation products, which are not extracted by chloroform from water.

Diazinon Metabolism in Resistant and Normal House Flies.—Diazinon was applied topically in this particular set of experiments, since the resistance data with house flies were all on this basis. Figure 1a shows the amount of Diazinon removed from the surface of the house flies with an acetone rinse, and the chloroform extractibles and inextractibles present within the house flies. The results with 4 μg./gm. showed the same relationships as with 30 μg./gm. At 0.5 hr. after treatment approximately 70% and 90% of the applied dosage was recovered (as combined surface and internal material) from the resistant and normal strain, respectively. It is unlikely that 30% of the Diazinon was excreted by the resistant strain within 0.5 hr.; since the dosages used (4 μg./gm. and 30 μg./gm.) rapidly immobilized the normal strain, whereas the resistant strain remained normally active, it may be that a portion of the topically applied dose was rubbed off by the resistant insects, particularly during the first hour after treatment.

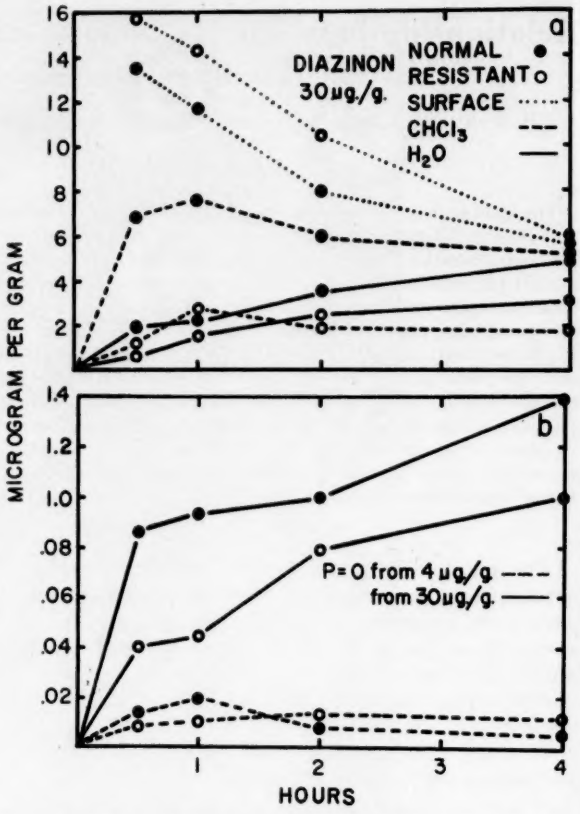

FIG. 1.—The fate of Diazinon topically applied to normal and resistant house flies. (a) Total surface material; and internal material fractionated into chloroform-extractibles ("$CHCl_3$") and inextractibles ("H_2O"). (b) Levels of P=O analog. Results expressed as μg. of Diazinon equivalents per gm. body weight. Treatment at zero time.

The level of chloroform-extractibles in the normal strain of house flies exceeded those in the resistant strain by a factor of 3 during the first hour after treatment. This differential cannot be attributed to extra detoxification in the resistant insects since the chloroform inextractibles were somewhat higher in the normal strain. It would therefore appear that the difference in penetration, possibly in conjunction with some surface loss, would explain the high chloroform-extractibles present in the normal fly. If a portion of the Diazinon were indeed removed by abrasion during the test period, the differences in penetration would be greater than is shown in figure 1a.

The levels of P=O analog in the normal and resistant strain at 4 μg./gm. and 30 μg./gm. dosages are shown in figure 1b. The level of P=O analog is consistently higher in the normal flies at the 30 μg./gm. dose, being about 2.5 times greater at 1 hr. At the 4 μg./gm. dose the difference

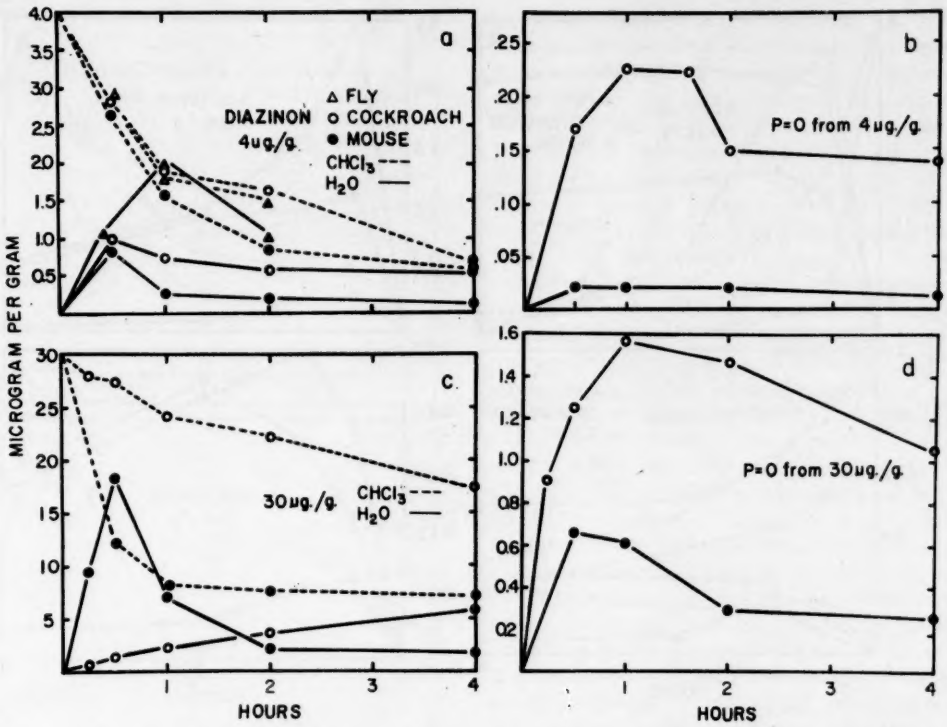

FIG. 2.—Metabolism of dimethoate injected into the house fly, American cockroach, and mouse. (a) and (c) Chloroform extractibles ("$CHCl_3$") and inextractibles ("H_2O"). (b) and (d) Levels of P=O analog. Results expressed as μg. of dimethoate equivalents per gm. body weight.

is small and appears to reverse after 2 hr. but this may not be significant.

Diazinon Metabolism in Insects and Mice.—For these and subsequent experiments, the organophosphates were injected. This method has the advantage that intrinsic biochemical differences are being studied and separated clearly from penetration effects. With topical studies it is difficult to decide if a difference between species is a reflection of penetrative or metabolic differences. It is better to evaluate these two factors separately.

With Diazinon, the low dose was applied to house fly, American cockroach, and mouse, but the high dose could be applied only to the cockroach and mouse, since the house flies were rapidly killed. After injection of Diazinon, the chloroform extractibles in the mouse decreased more rapidly than in the housefly or American cockroach (figs. 2a, 2c). The difference was very small at first with the low dose of 4 μg./gm., but substantial with the high dose of 30 μg./gm.: the mouse lost 22 μg./gm., the cockroach 6 μg./gm. These differences reflect the greater capacity of the mouse to degrade either Diazinon or its P=O analogue.

The chloroform inextractibles at the low dose were always lowest in the mouse. Therefore, in spite of the rather more rapid degradation than in the insect, the mouse could also dispose of the degradation products more readily (presumably by urination, although this assumption was not verified). At the high dose the chloroform inextractibles in the mouse showed a sharp peak at 0.5 hr. and at that time were 18 times higher in the mouse than in the cockroach. But by 2 hr. the level in the mouse was one-half that in the cockroach.

The levels of P=O analog of Diazinon which appeared in the cockroach and mouse are shown in figures 2b and 2d; the peak level appeared within 1 to 2 hours after treatment. The levels at both doses were markedly higher in the cockroach, and this difference was more extreme at the lower dose. The P=O analog constituted a rather constant part (3% to 7%) of the chloroform extractibles at any given time in both the cockroach and the mouse.

Acethion Metabolism.—Injected acethion was degraded very rapidly in the three animals studied. Degradation was particularly rapid in the cockroach and the mouse, in which 77%–88% of the injected dose appeared as chloroform inextractibles within 0.5 hr. after treatment (fig. 3a). As a corollary, the chloroform inextractibles in the house fly were substantially less than for the cockroach or mouse within the first hour after treatment. The sharp decline thereafter in chloroform inextractibles in the mouse again illustrates its capacity for rapid excretion.

The levels of P=O analog of acethion are shown in figure 3b. At 0.5 hours after treatment 6% of the chloroform extractibles appeared as the P=O analog in the case of the house fly, 8.4% for the cockroach and 4% for the mouse. These ratios remained rather constant throughout the test period. The decreasing difference in levels of P=O analog between the cockroach and house fly from 0.5 hr. onward follows a similar decrease in the difference of chloroform-extractibles.

Dimethoate Metabolism.—The mouse showed an exceptional ability to detoxify dimethoate: 89% of a 0.5

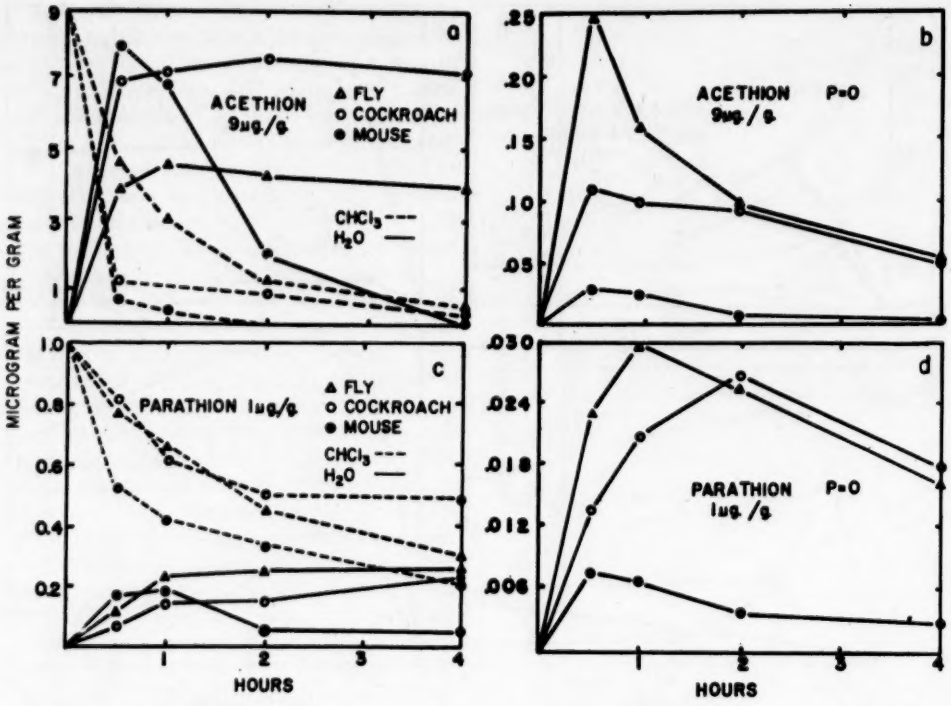

FIG. 3.—Metabolism of acethion and parathion by the house fly, American cockroach, and mouse. (a) and (c) Chloroform extractibles ("$CHCl_3$") and inextractibles ("H_2O"). (b) and (d) Levels of P=O analog. Results expressed as µg. of acethion or parathion equivalents per gm. body weight.

µg./gm. dose and 76% of a 30 µg./gm. dose appeared in the chloroform inextractibles within 0.5 hours after treatment (figs. 4a, 4b).

The P=O analog of dimethoate reached the highest levels in both the cockroach and mouse at about 0.5 or 1 hour after injection (fig. 4c). The P=O analog levels were approximately three times greater in the cockroach than in the mouse. The P=O analog represented about 3% of the chloroform extractibles in the mouse and 1.5% in the cockroach throughout the 4-hour period.

Parathion Metabolism.—With injected parathion the chloroform inextractibles were somewhat similar during the first 1.5 hours after treatment in insects and mice (fig. 3c). The levels of chloroform extractibles present in the mouse were consistently lower than those in the cockroach and house fly but the difference was relatively small (2-fold) and consistent. Once again the P=O analog (paraoxon) accounted for a fairly constant fraction of the chloroform extractibles; 5% in the house fly, 3% in the cockroach but only 1.5% in the mouse (fig. 3d). The most important fact, however, was that with this relatively nonselective compound there was a striking difference in the paraoxon levels in insects and the mouse (about 8-fold).

Toxicity.—Table 1 shows the LD_{50} of the compounds to the three animals. The 24-hour topical values for the insects give a good measure of the selectivity. It was considered desirable to show that the same selectivity pattern existed under the conditions used in this study, i.e., injection of the compounds. For the cockroach the injection data are substantially the same as the topical data. For the house fly, the toxic effect of the propylene glycol alone was severe, and most house flies were dead at 24 hr. At 4 hr. none were dead with propylene glycol alone, and it is therefore for this period that the LD_{50} by injection is shown. Naturally it requires more of a compound to kill an animal in 4 hr. than in 24 hr., therefore it is claimed for these data only that they indicate that the selectivity of these compounds is not markedly dependent upon route.

DISCUSSION.—The study on Diazinon-resistant as compared with normal flies indicated only a small difference in integument permeability (about 7% at both doses); about a 2.5-fold difference in Diazoxon level with both doses (at point of maximum difference); and about a 3-fold difference in the level of chloroform-extractibles. Although all of these operated in favor of the resistant strain, the differences are somewhat small compared to the 40-fold difference in LD_{50}. It should be emphasized here that the resistant flies showed no symptoms, while

Table 1.—Comparative LD_{50} values in µg./gm. for several organophosphates against insects and the mouse.

	House fly		American Cockroach		Mouse
Insecticide	Injected[a]	Topical[b]	Injected[b]	Topical[b]	Injected Intraperitoneal[b]
Acethion	15.0	9.4	375.00	700.0	1,280.0
Diazinon	4.0	1.5	0.75	2.0	40.0
Dimethoate	1.0	0.43	1.0	2.0	140.0
Parathion	1.5	0.9	0.9	0.9	5.5

[a] 4-hr. LD_{50}.
[b] 24-hr. LD_{50}.

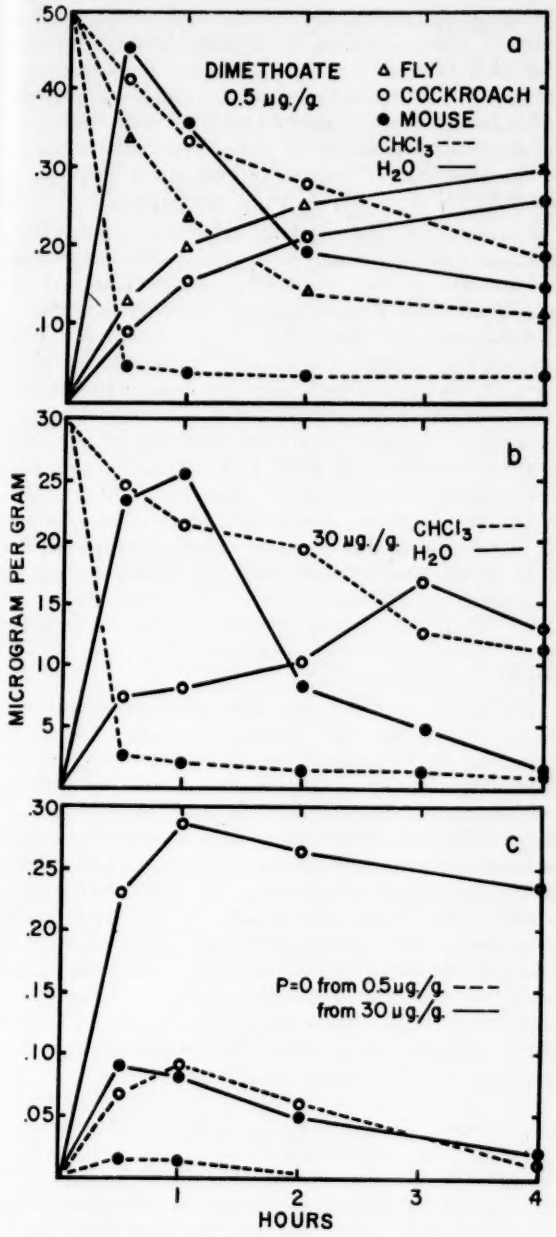

FIG. 4.—Metabolism of dimethoate by the house fly, American cockroach, and mouse. (a) and (b) Chloroform extractibles ("$CHCl_3$") and inextractibles ("H_2O"). (c) Levels of P=O analog. Results expressed as µg. of dimethoate equivalents per gm. body weight.

the normal strain of house flies displayed severe symptoms of organophosphate poisoning. At present the resistance cannot be reliably attributed to difference in penetration or metabolism of Diazinon. In order to determine the significance of these differences, metabolism at various dosage levels in a series of strains of varying resistance will have to be examined.

Before evaluating the other data, it is necessary to decide on what basis one can attribute selectivity to an observed metabolic difference. In a previous study on malathion (Krueger & O'Brien 1959) a striking difference in malaoxon levels was found: malaoxon (which is almost certainly the actual toxicant in malathion poisoning) achieved low and transient levels in the nonsusceptible mouse, but high and persistent levels in the two susceptible insects. This difference seemed sufficient to explain the toxicity difference.

In the present paper similar significant differences were found in the levels of P=O analogs for the selectively toxic compounds dimethoate, Diazinon and acethion (table 2). However, just such a difference was also found for the relatively nonselective compound parathion. In the house fly the 136-fold selectivity of acethion was associated with a 5-fold difference in P=O analog level; yet the 6-fold selectivity of parathion was associated with a 4-fold difference in P=O level. In the cockroach the 2-fold selectivity of parathion was associated with a 3-fold difference in P=O level, yet the 70-fold selectivity of dimethoate was associated with only a 2-fold difference in P=O analog level at the lower dose, or 3-fold at the higher dose. Therefore although it is true that selectivity is always accompanied by an increased P=O analog level in the susceptible species, it cannot be concluded that these phenomena are causally related.

A second hypothesis is that it is the level of total chloroform extractibles (i.e., principally the parent compound, with some P=O analog) which dictates toxicity. The evidence in favor of this hypothesis (table 2) is somewhat better. For example, low selectivity between insect and mouse was found with parathion for the house fly and cockroach and with acethion for the cockroach (toxicity ratios 6, 6 and 1.8) and correspondingly small differences of chloroform-extractibles between insect and mouse were found (ratios 1.6, 1.5 and 2.2). High selectivity was found with acethion for the house fly, and with dimethoate for the house fly and cockroach (toxicity ratios 136, 325 and 70) and correspondingly large differ-

Table 2.—Comparative levels of some metabolites in insects and the mouse 1 hour after poisoning.

		Ratios of Levels or of Toxicities			
		Parathion	Acethion	Diazinon	Dimethoate
		House fly			
$\frac{P=O \text{ fly}}{P=O \text{ mouse}}$		4.4	5.3	—	—
$\frac{CHCl_3 \text{ fly}}{CHCl_3 \text{ mouse}}$		1.6	5.6	1.1	7.7
$\frac{LD_{50} \text{ mouse}}{LD_{50} \text{ fly}}$		6.0	136	37	325
		American Cockroach			
$\frac{P=O \text{ roach}}{P=O \text{ mouse}}$	high dose	—	—	2.5	3.2
	low dose	3.0	3.3	11.5	2.3
$\frac{CHCl_3 \text{ roach}}{CHCl_3 \text{ mouse}}$	high dose	—	—	3.0	8.8
	low dose	1.5	2.2	1.2	11.0
$\frac{LD_{50} \text{ mouse}}{LD_{50} \text{ roach}}$		6.0	1.8	20	70

ences of chloroform extractibles were observed (ratios 5.6, 7.7, and 8.8–11.0). For the compounds parathion, acethion, and dimethoate there is therefore a good correlation between chloroform-extractible level and toxicity, but a poor correlation between P=O analog level and toxicity. The second hypothesis finds support in the case of these compounds.

It was at first disappointing to have to discard the simpler hypothesis that the oxygen analog level dictates toxicity. In fact it is highly probable that the oxygen analog level *at the target site* dictates toxicity; how well the gross level (as studied here) reflects the level at the target site will depend upon a multitude of factors, of which the most important variable, as far as the properties of the compound are concerned, is probably the liposolubility of the parent and its oxidation product. This property varies widely in the four compounds studied: for example, dimethoate is quite water soluble, whereas the others are substantially insoluble in water.

For Diazinon the chloroform extractibles were a very poor measure of toxicity. Although the selectivity was 37-fold for the house fly and 20-fold for the cockroach, the differences in chloroform extractibles between insects and the mouse were 1.1-fold for the house fly and 3.0- or 1.2-fold for the cockroach, depending upon dose. Now there is no particular reason why the selectivity of all compounds should have the same explanation. Can it be that in this case the P=O analog level is critical? Unfortunately the data are available only for the cockroach and are very dependent upon dose. Yet it seems significant that at the lower dose there was an 11.5-fold difference in P=O analog level between the insect and mouse; this difference is by far the largest found in this study or the previous study upon malathion (Krueger & O'Brien 1959). For Diazinon, therefore, the first hypothesis is very tentatively accepted: differences in P=O analog level are responsible for selectivity. This also appears to be true of the previous study on malathion, for which chloroform extractibles are poorly correlated with selective toxicity.

A special feature of Diazinon that makes its study difficult is that its degree of selectivity (20-fold for the cockroach and 37-fold for the house fly) is intermediate between what has been called in this paper low selectivity (1.8-fold up to 6-fold) and high selectivity (70-fold to 325-fold).

So far the selectivity discussed has been between insects and the mouse. The toxicity ratios, LD_{50} cockroach: LD_{50} house fly, for parathion, acethion, Diazinon and dimethoate are 1, 76, 1.8 and 4.6, respectively. The corresponding chloroform-extractible ratio are 1.1, 2.5, 0.9 and 0.7 and the P=O analog ratios are 1.5, 1.6, –, – (no figures are available for Diazinon and dimethoate in the house fly). Such data suggest that, as in malathion, metabolic differences are not principal factors in accounting for selectivity between insect species.

With dimethoate and acethion, the chloroform extractibles were generally lower in the mouse than in the insect, and this can mean only that the mouse degrades either the parent compounds or the P=O analogs more readily than the insect. Selectivity is thus probably caused by differences in ability to degrade, not to differences in ability to activate. In confirmation, the P=O analogs of malathion, dimethoate, and acethion are selectively toxic (Krueger & O'Brien 1959, Dauterman *et al.* 1959, O'Brien *et al.* 1958) although the selectivity is not so great as that of their parent compounds, probably owing to the "opportunity factor" (O'Brien 1959), *i.e.*, the delay in toxic action of phosphorothionates (caused by the necessity for their oxidation), which gives an opportunity for degrading enzymes to operate.

It is likely that the selective degradation of malathion, acethion, and dimethoate is due to high carboxyesterase and amidase activity, respectively, in the mammal. But both Diazinon and parathion are probably degraded by phosphatases (Aldridge 1953, Robbins *et al.* 1956). If the selectivity of Diazinon is caused by degradative differences, the mouse and cockroach (for instance) must differ in their capacity to degrade Diazinon but not in their capacity to degrade parathion. Different phosphatases must therefore be involved. It may be that for other selective organophosphates which have no carboxyester or carboxyamide groups, appropriate phosphatases are present in the mammal and not the insect, *e.g.*, for Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), ronnel, Dowco 109 (phosphoramidothioic acid, methyl-*O*(4-*tert*-butyl-2-chlorophenyl) *O*-methyl ester), and Chlorthion® (*O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate).

The findings in this paper must be accepted cautiously in view of the fact that whereas the LD_{50}'s used in the discussion were topical 24-hr. values (the usual figure), the treatments for metabolic studies were by injection (except in the brief study on resistance). These treatments were deliberate, inasmuch as it was desired to study metabolic differences unconfused by absorption differences. However, there are no good procedures for injecting house flies with apolar substances, and the propylene glycol which was used as a vehicle had a serious toxic action in the house fly. We cannot therefore exclude the possibility that the results for the house fly are partly artifactual. This criticism does not apply to the cockroach or mouse, for which the volume of propylene glycol was much smaller relative to body weight.

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DDT-Dehydrochlorinase III. Solubilization of Insecticides by Lipoprotein[1]

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ABSTRACT

Using egg yolk lipovitellin as a model system, the solubility of various organic insecticides is shown to be 10^4 to 10^5 times greater in lipoprotein than in water. This property can be exploited for the incorporation of water-insoluble compounds in enzyme systems as substrate levels. With lipoprotein-solubilized DDT as substrate and DDT-dehydrochlorinase as the enzyme, the initial rate may be made to vary as a linear function of enzyme or substrate concentration. The applicability of this solubilization method to kinetic analysis is also evident from determinations of reaction order and equilibrium. On the other hand, the presentation of substrate to enzyme in the form of relatively large droplets or crystals results in reaction rates complicated by factors involving surface area, viscosity, sorbtion phenomena, and enzyme denaturation. A discussion of solubilization techniques with physical and surface active materials is offered as a guide to metabolic studies of insecticides, *in vitro*.

The insolubility of modern pesticides in aqueous media has hindered biochemical studies of insecticide action. This paper presents some methods for the inclusion of toxicants in enzyme systems at substrate (or inhibitor) levels together with some criteria for the suitability of such systems to kinetic analysis. The enzyme DDT-dehydrochlorinase (DDTase) can serve as an example for such a discussion since its course of action can be followed by some modification of each of the methods to be described (Sternburg *et al.*, 1954, Lipke & Kearns 1959). These methods can be defined as follows: 1) Direct measurement, in which the change in concentration of some component of an enzyme system is continuously monitored during the course of the reaction and is expressed as a rate constant; and 2) terminal analysis, in which the levels of reaction products are determined after (rather than during) the incubation period. As an example of these methods, the reaction:

$$(p\text{-}ClC_6H_4)_2CH\text{—}CCl_3 \xrightleftharpoons{GSH} (p\text{-}ClC_6H_4)_2C{=}CCl_2 + H^+ + Cl^-$$

can be followed directly by measuring the rate of optical density change caused by olefin formation (Lipke & Kearns 1959) or the rate of acid production in the Warburg apparatus (Swift 1958). In the case of terminal analysis, the amount of DDE formed may be determined colorimetrically or spectrophotometrically after a reaction period of 90 minutes (Sternburg *et al.* 1954). It is evident that the method of direct measurement requires intimate contact between enzyme, substrate, and cofactor so that the maximum rate of reaction may be realized during the entire period of incubation. In the case of modern organic pesticides, some solubilization technique is indicated. These will be dealt with in some detail. For purposes of this discussion solubilization can be stated as that process which provides sufficient substrate for an enzyme system so that the initial rate of reaction is proportional only to enzyme concentration. None of the methods to be described render the substrate soluble in the strict physico-chemical sense, since the Tyndall effect may be observed in all instances; nevertheless, saturation of the enzyme with substrate can be achieved in these colloidal systems.

MATERIALS AND METHODS.—Pavement Marking Beads (3M "Superbrite" #119) were obtained from the Minnesota Mining Company, Minneapolis, Minnesota, and used according to Sternburg *et al.* (1954). Egg yolk lipoprotein and DDTase were prepared as described by Lipke & Kearns (1959). Crystalline bovine serum albumin was purchased from the Armour Co., Chicago, Illinois. Lecithin, digitonin, and sodium taurocholate were obtained from Nutritional Biochemicals Corp., of Cleveland, Ohio. Whenever possible, Eastman Spectral Grade solvents were used and all solvents were redistilled before use. For direct spectrophotometry, saturated solutions of DDT in various solvents were agitated at 40° with 5 volumes of water in a mechanical shaker. Aliquots of the aqueous layer were incubated with enzyme and glutathione as described for the lipoprotein system. Oil emulsions of DDT were also prepared in a mechanical shaker with 1 part of olive, peanut, or soybean oil to 3 of aqueous buffer and 0.1 part of glass beads. Synthetic surfactants were provided through the courtesy of the Atlas Powder Co. of Wilmington, Delaware. These materials were decolor-

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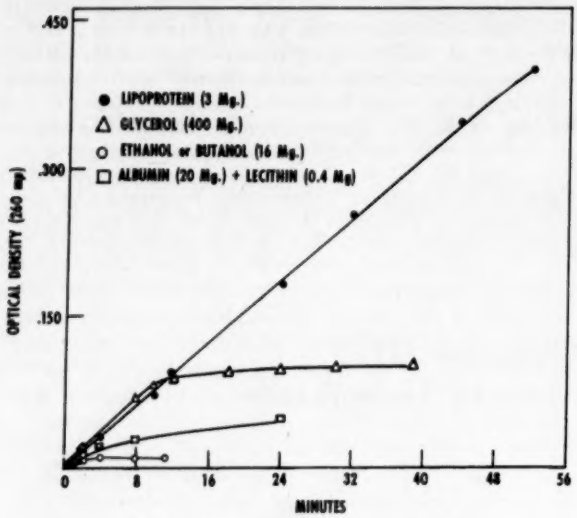

FIG. 1.—Effect of solubilizing agents of DDTase as measured by direct spectrophotometry. Method of Lipke & Kearns (1959), final volume 3.5 ml.

ized by passage through charcoal columns. Purified aldrin, dieldrin, heptachlor and lindane were furnished by the Shell Chemical Company. Pyrethrin I was purified by Dr. S. C. Chang by chromatography on celite-alumina according to an unpublished procedure. TDE was furnished by the Rohm and Haas Co. Other DDT derivatives were synthesized by Dr. J. G. Sternburg from published methods.

RESULTS AND DISCUSSION.—The ability of alcoholic solutions of insecticides to form fine suspensions has frequently been exploited in biochemical and physiological studies of drug action. From 10 to 100 μl. of an ethanolic or glycol solution of insecticide are added to about 4 ml. of enzyme preparation (Hayano & Dorfman 1953, Sternburg *et al.* 1954, Young 1959). Where direct spectrophotometry is feasible, the alcohols and glycols have the advantage of low optical density in the ultraviolet region but definite disadvantages accompany the use of mixed solvent systems. Although an enzyme has been described by Hanrahan *et al.* (1954) that retains activity in 80% ethyl ether, the majority undergo extensive denaturation when incubated at physiological temperatures with traces of organic solvents (Sternburg *et al.* 1954, Swift 1958). This effect is particularly noticeable with highly organized systems such as cell particulates or with purified enzyme preparations. Figure 1 shows the effect of 20 μl. of ethanol and butanol-DDT suspensions on partially purified DDTase in a volume of 3.5 ml. Still lower rates of reaction were observed with dimethylformamide and acetonitrile. It can be seen also that the rate with DDT-saturated glycerol is constant during a 12-minute interval during which an optical density change (ΔOD_{260}) of 0.10 occurs. That the rate was maximal for this period can be observed from a comparison with lipoprotein-solubilized DDT, to be discussed subsequently. Although the glycerol system appears satisfactory at first glance, an optical density charge of this order is not sufficient for studies with highly active preparations of DDTase. Additional increments of substrate in the form of glycerol-DDT increase the viscosity of the system and reduce the reaction rate. O'Brien (1956) has observed interference by ethanol in studies of organophosphate inhibition of choline esterase.

Direct spectroscopy has been used in the Illinois laboratory with house fly (*Musca domestica* L.), hornworm, and bean beetle DDTase subjected to varying degrees of purification. The method depends on the presence of a well-defined absorbtion maximum or minimum on the part of the substrate, product, or coupled indicator system. Direct spectrophotometry may be possible with crude enzyme preparations if spectral regions can be chosen where interference from tissue components is at a minimum. The presence of turbidity or high absorbance owing to the solubilizing agent may occasionally be overcome by increasing the sensitivity of the system with the photomultiplier attachment and the thermostatically controlled cell compartment. The latter device can maintain an elevated temperature at which reaction rates are greater than in the conventional cell compartment. In view of the information that can be obtained pertaining to reaction mechanisms and kinetics, as well as its simplicity and rapidity, the time spent in the development of a direct spectrophotometric method for a given enzyme system appears to be fully justified.

In the case of terminal analysis, considerably greater choice of solubilizing agents is available than in direct spectrophotometry. All the solubilizing expedients described under direct spectrophotometry are, of course, applicable to conventional methods of insecticide analysis, although the occasional need for sample "clean-up" may be indicated. Since terminal analysis permits vigorous shaking of the enzyme system with no requirement for optical clarity, use may be made of fine suspensions of insecticides in oil emulsions. Some stable, highly refined oil emulsions are now available commercially.[3] With respect to the stabilization of emulsions, the ability of the enzyme system to withstand detergent action governs the use of natural and synthetic surface active materials with such emulsions. With the exception of the alkali metal salts of the saturated fatty acids, the naturally occurring surfactants available commercially absorb strongly in the ultraviolet region but may be suitable for terminal analysis. These surfactants include lecithin, bile salts, (such as sodium taurocholate) and digitonin (Hubbard 1956, Ekwall *et al.* 1951). The use of these emulsifiers has been limited by the frequency with which enzyme denaturation has been encountered (Sternburg *et al.* 1954, Sanborn 1956). It should be emphasized that the solubilizing action of these materials may be sufficient to stabilize an insecticide-oil emulsion when added in trace amounts, 0.01% or less, at which level little inactivation may occur.

Some of the shortcomings of the materials discussed above can be circumvented by the use of purely physical suspensory media. Sternburg *et al.* (1954) introduced the insecticide-coated glass bead. This method requires no elaborate equipment and is perhaps the most adaptable of the current devices. The beads should be washed and neutralized before use. A substitute is available in the form of a glass powder made by grinding pyrex glass in a

[3] Ediol, Schenley Co., New York, and Lipomul, Upjohn Co., New York.

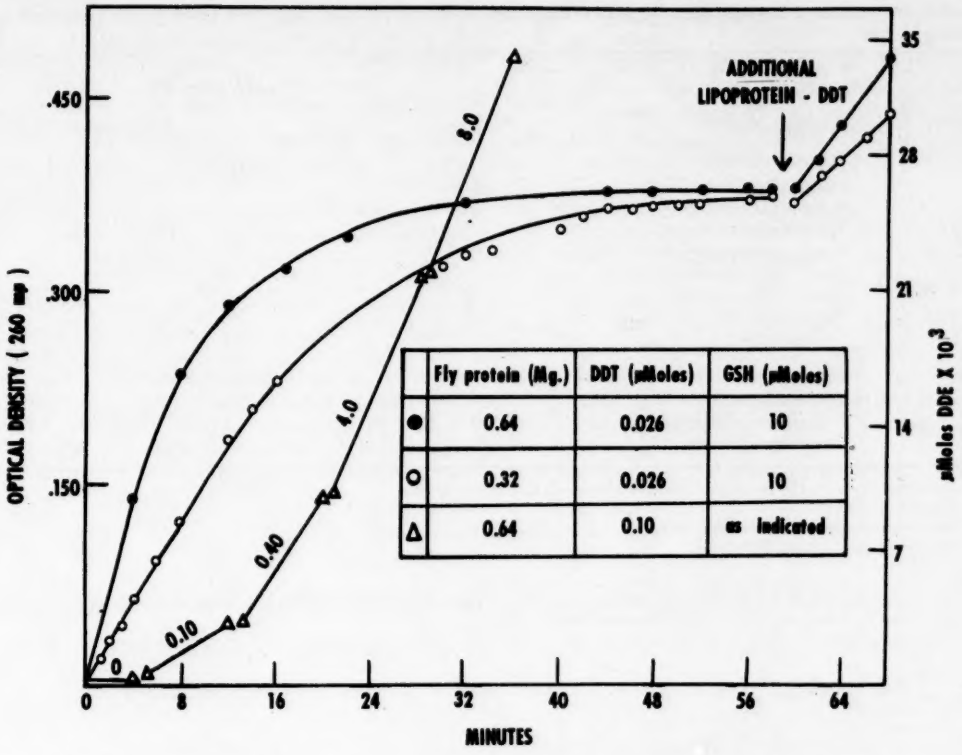

FIG. 2.—Effect of enzyme and glutathione concentration on reaction rate and equilibrium of DDTase as determined with lipoprotein-solubilized DDT (Lipke & Kearns 1959).

mortar (Neeman *et al.* 1957). Inhalation of this material should be avoided. The bead procedure has given satisfactory results with crude enzyme systems but is unsuited to studies requiring the determination of initial rates. This disadvantage originates in the phenomenon of sorbtion of dilute enzyme preparations on the beads, thus purified DDTase fractions of high specific activity show a nonlinear response of activity to enzyme concentration. This effect is presented in table 1. It can be seen that specific activity does not remain constant with different concentrations of DDTase since DDE production is not a direct function of enzyme concentration. This deviation from linearity becomes more pronounced with greater dilution of enzyme and enzyme of increasing purity. An effect of this nature is frequently ascribed to increasing dilution of an inhibitor present in the enzyme extract (Scott & Cohen 1953). That this is not the case with the bead assay for DDTase is evident from figure 2 where it is shown by the method of Lipke & Kearns (1959) that a ΔOD_{260} of 0.135 in the lipoprotein system is maintained for 5 minutes with 0.64 mg. of enzyme preparation in 3.5 ml., whereas the ΔOD_{260} for this time interval with half as much enzyme preparation is 0.066. Thus, the data show that in a suitable system the response of enzyme activity to enzyme concentration is linear, indicating that no inhibitor is present in the enzyme extracts. It should also be noted that the equilibrium of the DDTase reaction lies far to the right, and DDT-breakdown is essentially complete at the time of addition of a second increment of substrate (Lipke & Kearns 1959). Some improvement in linearity with the bead assay may be obtained by the addition of sufficient serum albumin to equate the total protein in all the vessels. That absorbtion occurs with substrate as well as enzyme is also apparent from table 1. Pretreatment of the beads with DDT 48 hours rather than 1 hour before incubation with enzyme resulted in an 80% inhibition of the reaction. Bruce (1957) has reported that dilute agar suspensions of DDT may be formulated that are suitable for DDTase assays with crude preparations.

The stabilizing effect of proteins on lipid-water emulsions has been exploited by investigators concerned with enzymatic transformations of sterols (Swell & Treadwell 1950). Serum albumin is the most widely used protein because of its availability, purity and protective effect on labile enzyme systems. Chang & Kearns (1958) have studied the oxidation of pyrethrins by flight muscle

Table 1.—Effect of enzyme concentration on activity of DDTase with glass bead assay.[a]

PROTEIN (MG.)	μG. DDE/60 MIN.	SPECIFIC ACTIVITY[b]
4.0	426	106
2.0	326	163
1.0	287	287
4.0[c]	82	21

[a] Procedure according to Sternburg *et al* 1954. Enzyme preparation fraction 1, $\Delta_{260} = 0.13$ (Lipke and Kearns 1959).

[b] μg. DDE/60 min./mg. protein.

[c] Enzyme, glutathione and buffer added to beads coated with DDT 48 hours previous to incubation. Other values with beads coated 1 hour previous to incubation.

Table 2.—Solubilization of insecticides by ether-extracted and unextracted egg yolk lipoprotein prepared according to Lipke & Kearns (1959).

Insecticide	Assay Method[a]	Solubility, μMoles/Ml.		
		Water[b]	Lipoprotein	
			Unextracted[c]	Extracted[d]
DDT	Colorimetric[e]	1×10^{-4}[f]	12	3
TDE	Spectrophotometric[g]	—	—	5
p,p'-I DDT	Spectrophotometric[g]	—	—	5
p,p'-Br DDT	Spectrophotometric[g]	—	—	1
p,p'-F DDT	Spectrophotometric[g]	—	—	50
p,p'-CH_3 DDT	Spectrophotometric[g]	—	—	20
Methoxychlor	Spectrophotometric[g]	—	—	22
DDE	Colorimetric[e]	—	14	4
Lindane	Colorimetric[h]	3×10^{-2}[i]	21	—
Heptachlor	Colorimetric[j,k]	—	20	—
Aldrin	Colorimetric[k,l]	—	22	—
Dieldrin	Colorimetric[k,m]	$<5\times10^{-4}$	3	—
Rotenone	Spectrophotometric[g]	4×10^{-4}[i]	—	13

[a] Colorimetric procedures preceded by removal of interfering materials by chromatography.
[b] Approximate solubilities at 25°.
[c] Unextracted lipoprotein concentration; 100 mg./ml.
[d] Extracted lipoprotein concentration; 20 mg./ml.
[e] Schechter *et al.* (1945).
[f] Babers (1955).
[g] Values with spectrophotometric method obtained by difference spectra of lipoprotein with and without insecticide at wave length of maximum absorbance. Molecular extinctions from Sternburg *et al.* (1954) and Gunther & Blinn (1955).
[h] Schechter & Hornstein (1952).
[i] Brown (1951).
[j] Polen & Silverman (1952).
[k] Analysis courtesy of Dr. N. Gannon.
[l] O'Donnell *et al.* (1954).
[m] O'Donnell *et al.* (1955).

mitochondria and find that these insecticides can be solubilized at substrate levels with bovine serum albumin. No hydrolysis of the ester was effected by the albumin. A group of lipoproteins of low lipid content is occasionally available in the form of lyophilized Cohen fractions from processors of gamma globulin. Nason & Lehman (1955) found that gamma globulin rendered the tocopherols sufficiently soluble to make their incorporation in enzyme systems possible.

Lipke & Kearns (1959) have described the preparation of an egg yolk lipoprotein which is high in lipide content, stable, and optically clear following ether extraction. Table 2 presents the solubility data for various insecticides. The suitability of this material for kinetic studies is shown in figure 2, where the initial rate of DDE formation at different concentrations of enzyme and coenzyme and the equilibrium of DDTase are presented. Solubilization by egg yolk lipoprotein can not be used indiscriminately, for this material has a strong esterolytic action (Chang & Kearns 1958) on the pyrethrins. Since esterase action is markedly reduced, but not entirely eliminated, by ether extraction, methods can probably be developed for the complete inactivation of the egg yolk esterase. The elimination of esterase activity would extend the use of this material to organic phosphate and substituted carbamate insecticides. The use of lipoprotein carriers may also benefit physiological investigations with perfused organ systems.

In the case of the DDT-resistant house fly, the equilibrium of the DDTase system and the presence of tissue lipoprotein capable of solubilizing DDT at specific target sites may be combined to produce rapid and complete degradation of toxicant in a manner analogous to the situation observed *in vitro* (Miyake *et al.* 1957). The effect of solubilizing agents, such as lipoproteins, may be in the acceleration of detoxicating systems, thus the presumed storage function of these conjugated proteins (Wiesmann 1957) remains to be verified by experimentation.

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Syntheses of the Aliphatic Deuterium Analogs of DDT and TDE and Their Toxicity and Degradation When Applied to Adult House Flies[1]

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ABSTRACT

The deuterated analogs of DDT (1,1,1-trichloro-2d,2,2-bis (*p*-chlorophenyl) ethane) and of TDE (1,1-dichloro-2d,2,2-bis (*p*-chlorophenyl) ethane) were synthesized and used to measure isotope effect on absorption spectra, insecticidal action, and chemical stability. Since deuteration gave little change in toxicity or degradation, these properties evidently involve the electronic structure of the molecule, not mass effects.

A critical structural topography for DDT toxicity has been postulated by Gunther *et al.* (1954). Homologs and analogs having polarizability and molecular architecture similar to DDT are expected to have similar biological activity. The introduction of such an isotopic atom as deuterium should leave essentially unchanged all properties of the molecule associated with electronic structure and force fields, but should affect properties depending upon mass, such as vibrational frequencies and moments of inertia. If the key to DDT toxicity is an electron pattern that physically fits a susceptible site, then either DDT or d-DDT and either TDE or d-TDE would be expected to be equitoxic at that site. The limitations of such a concept have been emphasized by Stringer *et al.* (1955).

Hennessy & O'Reilly (1956) proposed that DDT exerts a toxic effect through a chemical reaction involving, as a first step, *in vivo* removal of the aliphatic hydrogen to form a free radical. Substitution of deuterium for hydrogen has provided a valuable tool for the determination of reaction mechanisms. Three factors contribute to a generally lower reactivity of C—D bonds than C—H bonds: (1) difference in free energy which is mainly a difference in zero-point energy, (2) effect of difference in mass on the velocity of passage over the potential-energy barrier, and (3) nonclassical penetration of the energy barrier by a "tunnel effect" (Wiberg 1955, Bell *et al.* 1956). Although isotope effects for H/D as high as 14.5 have been reported, reactions in which isotope effects are greater than 4 have involved H or D as positive ions. Those reactions with isotope effects less than 3 involve bond breaking with H or D as a free radical or migration of H or D through a hydride shift (Collins *et al.* 1959). If the rate-determining step for toxicity involves a free-radical reaction, then DDT might be up to three times as toxic as d-DDT.

Antithetic to the hypothesis of Hennessy and O'Reilly, Perry & Buckner (1957) postulated removal of aliphatic hydrogen from the tertiary carbon as the first step in detoxication of Dilan (1 part 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts 1,1-bis(*p*-chlorophenyl)-2-nitrobutane) and perhaps DDT, by resistant house flies (*Musca domestica* L.). Their concept was a necessary modification of the theory of Kearns and coworkers that DDT resistance in house flies is due to an increase in DDT-dehydrochlorinase (Sternburg *et al.* 1950, 1953, 1954). In enzymatic reactions, deuteration reduced activity about 30% with acetyl choline (Erlenmeyer & Lobeck 1937), succinic acid (Erlenmeyer *et al.* 1936, Sonderhoff & Thomas 1937, Thorn 1951), formate (Kuchinskas *et al.* 1959), and acetate (Sonderhoff & Thomas 1937). Even greater isotope effects were found in utilization of methanol (du Vigneaud *et al.* 1951, Rachele *et al.* 1954) and methionine (Rachele *et al.* 1955).

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If d-DDT is degraded to DDE more slowly than DDT *in vivo*, and if dehydrochlorination is the cause of resistance, d-DDT should be more toxic than DDT to resistant flies.

Deuterium is the isotope of choice; it is inexpensive and, unlike many radioactive isotopes, it is stable and available in high isotopic purity. Deuterium is usually considered a satisfactory tracer for organic compounds when the reactions that such a labeled group undergoes do not involve cleavage of the C—H bond. However, when Verly *et al.* (1952) administered $C^{14}H_2DOH$ to rats and then isolated choline, the ratio of D to C^{14} in the choline was only 22% of that in the labeled methanol. This might have resulted from a selection in isotopic molecules, not atoms. Although deuterium has been used as a tracer in such large, biologically active molecules as steroids and fatty acids, the possibility of an effect on valence angles seems to have been dismissed.

This report concerns the syntheses of deuterated DDT and TDE and isotopic effects on: (1) physical properties such as melting point, appearance, and absorption spectra; (2) toxicity to susceptible and resistant strains of house flies; and (3) dehydrochlorination in alkali and in house flies.

SYNTHESIS.—In outline form the three steps employed were:

$$\text{I. } (CH_3CO)_2O + LiA1D_4 \rightarrow 2\,CH_3CD_2OH$$

$$\text{II. } 2\,CH_3CD_2OH + 2\,Cl_2 \rightarrow CCl_3CD(OH)OCD_2CH_3$$

$$\xrightarrow{H_2O} CCl_3CDO$$

$$\text{III. } CCl_3CDO + 2\,C_6H_5Cl \xrightarrow{H_2SO_4} CCl_3CD(C_6H_4Cl)_2$$

In the first step, α-d_2-ethanol was prepared by the method of Shiner (1953). The product, distilling at 76° to 80°, yielded 4.0 ml. (89%).

The second step was adapted from the procedure of Pearce & Jensen (1953). To minimize contamination from stopcock grease, 14/20 joint semi-micro apparatus was used without lubrication. α-d_2-Chloral was prepared from α-d_2-ethanol by slowly adding 20–25 gm. (0.28–0.35 M) of chlorine through a washing bottle containing concentrated sulfuric acid into a 50-ml. three-necked pear-shaped flask containing 4 ml. of magnetically stirred α-d_2-ethanol. Two days of chlorination at room temperature and 2 more days at reflux temperature gave a partially crystalline chlorinated product. About half the α-d_2-ethanol was wasted in hemiacetal formation. The deuterated chloral hemiacetal was not isolated.

The last step is a semi-micro version of the Ziedler synthesis of DDT. Deuterated DDT was prepared by adding 4 ml. (0.035 mole) of monochlorobenzene to the chlorinated products, cooling the mixture in an ice bath, and then adding, dropwise and with stirring, a mixture of 10 ml. of 30% fuming sulfuric acid and 30 ml. of reagent-grade sulfuric acid. The reaction mixture was packed in ice, stirred with a glycerol-lubricated precision-bored glass stirrer for 4 hours, and then poured onto 200 gm. of crushed ice. The products were extracted twice with 200 ml. of sulfuric acid-washed, redistilled (66° to 73° C. fraction) Skelly B petroleum either. Evaporation of the petroleum ether yielded 5.6 gm. (0.016 mole) of crude d-DDT. The d-DDT was recrystallized twice from ethanol to obtain 0.8 gm. of needles melting at 101°. The mixed melting point with DDT (m.p. 103°) was 102°.

Dachauer & Hennessy (1958) concurrently synthesized d-DDT by another route which probably lost less deuterium and offered fewer chances for hydrogen-deuterium exchange. A sample generously supplied by them had the same chemical and biological properties.

d-TDE was synthesized by the same procedure as d-DDT except that chlorine was added for only 12 hours at room temperature and then for 3 hours at reflux temperature. This gave chlorinated products (d = 1.44) containing deuterated dichloroacetaldehyde. The Ziedler condensation of these products yielded 5.6 gm. (0.018 mole) of crude d-TDE. Recrystallizing twice from ethanol yielded 1.78 gm. of plates that melted at 106.5° to 109.5°. TDE and its mixtures with d-TDE also had this melting point.

To confirm the identification of the products, 50 mg. samples of DDT, d-DDT, TDE, d-TDE, and DDE were added to 5 ml. of 10% methanolic sodium methoxide. After 24 hours the methanol was evaporated on a water bath; then 5 ml. of water was added to the sodium methoxide residue. The aqueous suspension was extracted twice with 2 ml. of petroleum ether (b.p. 40° to 44° C.). The petroleum ether extract was washed with 5 ml. of distilled water. The petroleum ether was evaporated to yield the dehydrochlorinated derivatives with the following melting points: from DDT, 84°; d-DDT, 79°; TDE, 55° to 60°; d-TDE, 58° to 61°. DDE, former m.p. 87°, melted at 83° after this process; pure dehydrochlorinated TDE should melt at 63° to 65°. These dehydrochlorinated derivatives were dissolved in 0.5 ml. of carbon disulfide, and the infrared spectra were obtained by using a 0.4 mm. potassium bromide cell in a Perkin-Elmer Model 21 spectrophotometer. The spectra, shown in fig. 1, indicate that the deuterated materials dehydrochlorinated to the same respective products as did DDT and TDE. The deuterated analogs gave typical colors of DDT and TDE with Schechter-Haller analyses (Sternburg *et al.* 1950). The chemical purity, then, of the deuterated analogs seems qualitatively confirmed.

ABSORPTION SPECTRA.—Deuteration left the ultraviolet spectra unaltered; this is to be expected when the electron configuration remains unchanged. With a Beckman Model DK-2 spectrophotometer, cyclohexane solutions, 10 mg. per ml., showed strong absorption peaks at 221 and 236 mμ for DDT and d-DDT, with weak peaks at 259, 266, and 276 mμ. Both TDE and d-TDE had strong absorption peaks at 219 and 231 mμ, with smaller peaks at 261, 268, and 276 mμ.

The same spectrophotometer was used to measure near-infrared spectra from 0.8 to 3.5 μ. In this region almost all the absorption bands arise from molecular vibrations involving C—H stretching. Strong absorption peaks were found in DDT, d-DDT, TDE, d-TDE, and DDE at 1.12, 1.65, 2.12, 2.17, 2.46, 3.23, and 3.29 μ; these absorptions evidently involve the *p*-chlorophenyl groups. DDT has only one aliphatic hydrogen. Since it is tertiary hydrogen, an extremely weak fundamental vibration for aliphatic hydrogen is expected near 3.46 μ (Brügel 1957). Resolution is reduced in quartz above 2.8

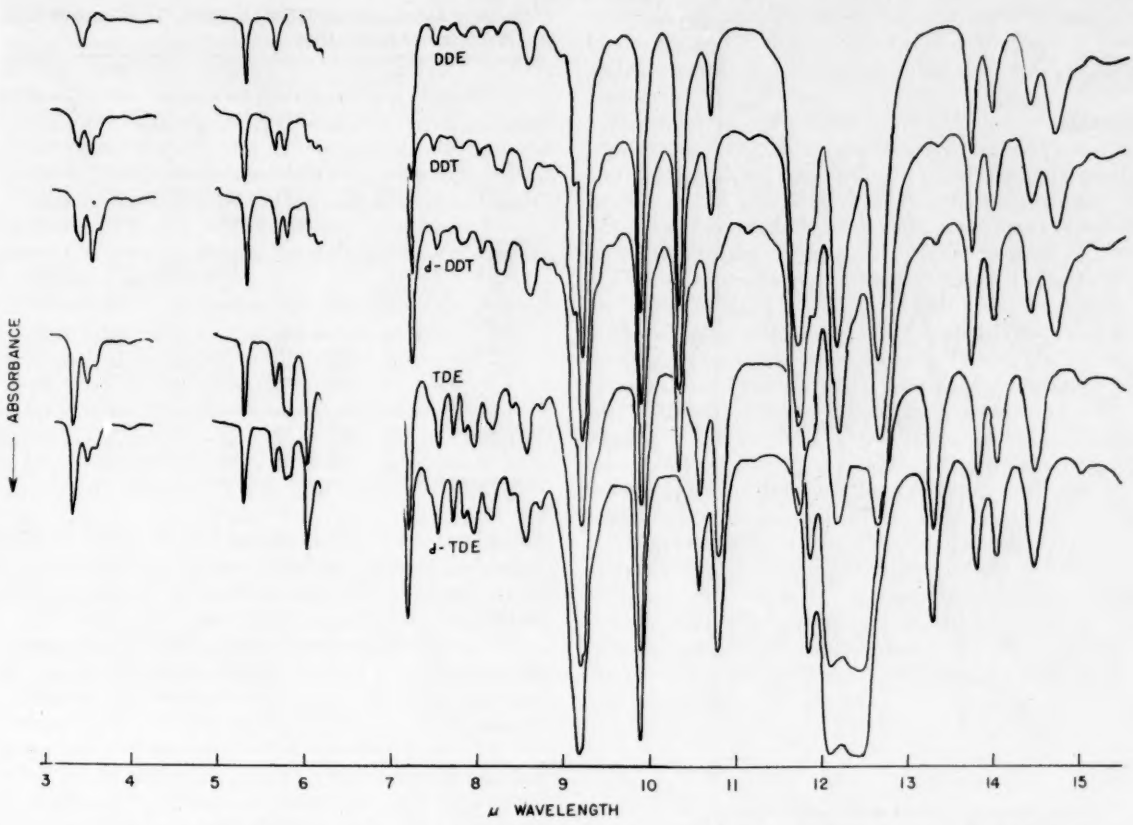

FIG. 1.—Infrared absorption spectra of dehydrochlorinated products of DDT, d-DDT, TDE, and d-TDE.

μ; so a high sensitivity setting was required to detect this bond. With the wavelength scale expanded and with the sensitivity at its highest setting, a peak for tertiary aliphatic C—H was found in DDT and TDE at 3.42 μ (fig. 2). DDE has no absorption at this peak; d-DDT has a slight peak, which suggests that 14% hydrogen analog may have contaminated the d-DDT. TDE has two tertiary aliphatic hydrogen atoms, one of which deuterium replaced in d-TDE. The area of the peak at 3.42 for d-TDE was 53% of the area for TDE. This suggests that 6% of d-TDE is contaminated with the hydrogen analog. Small differences in spectra were found at 1.74 μ, which seem to be the first overtones of tertiary C—H vibration. Deuterated compounds, showed weak peaks at 2.32 μ which may represent first overtones of fundamental tertiary C—D vibration.

The infrared absorption spectra (fig. 3) were obtained with a Perkin-Elmer Model 21 spectrophotometer using a sodium chloride prism. The spectra of nondeuterated DDT and TDE are in close agreement with those obtained by Downing *et al.* (1946) for *p,p'*-isomers; absorptions for *o,p'*- or *m,p'*-isomer and DDE are absent. The synthetic method evidently gave high isomeric purity. In the deuterated analogs the expected fundamental stretching vibration of C—D bonds was found at 4.50 and 4.65 μ. The C—H peak was found at 3.42 μ in DDT and TDE, and to some extent in d-TDE, as expected. A strong peak was present at 10.54 μ in d-DDT but not in DDT; DDT had peaks at 11.20 μ and at 12.39 μ, which were entirely absent in d-DDT. Their absence in d-DDT, contrary to the C—H peak in the near-infrared spectra, suggests high isotope purity for d-DDT. The peaks at 9.18 μ and 9.85 μ due to *p*-chlorophenyl were, of course, unchanged.

TDE had peaks at 11.39, 11.80, and 12.50 that were absent in d-TDE; d-TDE had peaks at 10.17, 11.07, 11.95, and 12.77 that were not found in TDE. This suggests a high isotope purity in d-TDE. The bands of medium intensity at 10.42 μ and 10.64 μ found by Dachauer and Hennessy (1958) in d-DDT and d-methoxychlor were very weak in d-TDE.

TOXICITY.—The DDT, d-DDT, TDE, and d-TDE were tested concurrently on skim-milk-fed, 3-day-old house flies of three strains: N, a susceptible strain originating from the National Association of Insecticide and Disinfectant Manufacturers' (NAIDM) strain and never exposed to DDT; LDD, a strain formerly of moderate resistance to several chlorinated hydrocarbons but unexposed to insecticides for at least six generations; R, a strain originating from the Orlando DDT-resistant strain with resistance maintained by exposing adults of each generation to a residue of DDT. Six dosages of each of the test insecticides were applied in 0.9 μl. of acetone to the thoraxes of 99,500 flies in groups of 250 with a micro-applicator. Dosages were geometric progressions to the base 2 and were selected to give nearly equal distribution of tests above and below 50% kill. Flies were reared by standard procedure (CSMA 1955) and were

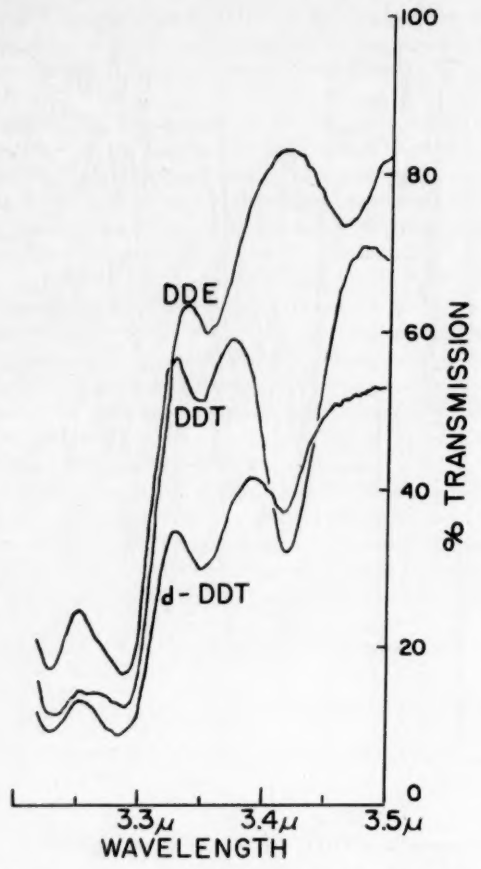

FIG. 2.—Near-infrared absorption spectra of DDT, d-DDT, and DDE determined with 66 mg./ml. of carbon tetrachloride using a Beckman DK-2 spectrophotometer with a tungsten lamp and lead sulfide detector using a 1 cm. quartz cell, an expanded wavelength scale, 0% to 100% transmission, period set at 0.6 sec., and sensitivity set at 312.

treated under carbon dioxide anesthesia, then held in screened 1-pint paper cans at 25° C. and 70% relative humidity in continuous light. Mortality was counted after 24 hours; flies exhibiting movements were considered alive.

Probit analyses of mortality data were calculated in the Cornell Computing Center of Cornell University with their usual modification of the method of Finney (1952) in which they fit the provisional line by least squares rather than by eye. The results are given in table 1. Since the slopes of the log-dosage probit lines were not parallel, a simply toxicity ratio between analogs cannot be assessed. At the LD-50 values of N males, for example, DDT was more toxic than d-DDT, but at the LD-95 dosages d-DDT was the more toxic. If survival depends upon the ability of a treated fly to remove the aliphatic hydrogen or deuterium to detoxify DDT, the deuterated analogs might be expected to have the steeper slopes. Dosage-mortality slopes actually were steeper for d-DDT than for DDT on both sexes of N flies and on males, but not on the females, of LDD flies. However, the deuterated analogs were just as ineffective on resistant flies. Applications of 0.7 μg. of DDT and d-DDT, each to 3,000 flies,

Table 1.—Toxicity of DDT d-DDT, TDE, and d-TDE to 3-day-old adult house flies.

TREATMENT	MICROGRAMS PER FLY WITH FIDUCIAL LIMITS FOR 95% PROBABILITY		LOG-DOSAGE PROBIT LINE SLOPE ± s.d.
	LD-50	LD-95	
N males:			
DDT	0.040 (0.034–0.047)	0.795 (0.636–0.993)	1.27 ± 0.07
d-DDT	.046 (.043– .049)	.276 (.255– .299)	2.11 ± .07
TDE	.380 (.363– .398)	1.21 (1.13 –1.31)	3.26 ± .10
d-TDE	.348 (.334– .363)	.858 (.805– .914)	4.20 ± .16
N females:			
DDT	0.145 (0.138–0.153)	0.694 (0.622–0.774)	2.42 ± 0.09
d-DDT	.116 (.112– .121)	.415 (.389– .443)	2.98 ± .07
TDE	.707 (.679– .736)	2.04 (1.89 –2.20)	3.57 ± .11
d-TDE	.721 (.694– .750)	1.92 (1.78 –2.06)	3.87 ± .12
LDD males			
DDT	0.081 (0.073–0.089)	0.671 (0.577–0.779)	1.79 ± 0.09
d-DDT	.070 (.065– .075)	.308 (.279– .341)	2.55 ± .11
TDE	.383 (.368– .398)	1.11 (1.03 –1.20)	3.55 ± .10
d-TDE	.421 (.405– .439)	.978 (.902–1.06)	4.50 ± .19
LDD females:			
DDT	0.188 (1.79 –0.198)	0.734 (0.668–0.806)	2.78 ± 0.09
d-DDT	.148 (.140– .156)	.644 (.583– .712)	2.57 ± .09
TDE	.742 (.708– .779)	2.04 (1.84 –2.25)	3.75 ± .14
d-TDE	.880 (.807– .960)	2.98 (2.39 –3.72)	3.10 ± .20

killed less than 0.1%. TDE and d-TDE, at 0.7 μg. per fly, killed less than 1% of 800 females and between 2% and 3% of 800 males. Because most of the R flies are immune to DDT in acetone, an LD-50 cannot be measured.

If successive treatments of equal dosages are considered as paired tests, a 1:1 null hypothesis for higher mortality can be used to determine whether the analogs are equitoxic. Since toxicity differences above 90% and below 10% are based on small numbers of flies and are more subject to errors, only those treatments giving between 10% and 90% kill were compared. The slopes were steeper for the TDE analogs than for DDT and d-DDT, and this resulted in fewer comparisons of TDE analogs. The data, summarized in table 2, indicate that under these conditions d-DDT was more toxic than DDT in a significant number of tests; TDE and d-TDE were equitoxic. Data of table 1 suggest that this difference between d-DDT and DDT is slight.

DEHYDROCHLORINATION.—Since a carbon-hydrogen bond has a lower activation energy than a carbon-deuterium bond, DDT or TDE would be expected to dehydrochlorinate more readily than the deuterated analogs. This was checked by adding twelve 70-μg. samples of each insecticide to 5 ml. of alkaline solutions (1:1 absolute ethanol/ 2% sodium hydroxide). After standing 60 minutes at 23°, dehydrochlorination was stopped by acidifying the solution with 5 ml. of 6 N sulfuric acid. The

Table 2.—Paired comparisons of isotopic analogs of DDT and TDE.

TEST	FREQUENCY OF HIGHER KILL IN PAIRED TESTS		χ^2
	DDT	d-DDT	
N males	3	13	6.3[a]
N females	3	15	8.0
LDD males	1	13	10.3
LDD females	1	15	12.3
	TDE	d-TDE	
N males	4	6	0.4
N females	6	8	0.3
LDD males	6	4	0.4
LDD females	4	2	0.6

[a] Above 3.8 is a significant difference.

ethanol was removed on a rotary film evaporator, and the aqueous residue extracted once with 50 ml. of ethyl ether. The ether was then evaporated, and the residues were analyzed by the Schechter-Haller method as modified by Sternburg *et al.* (1950). After 60 minutes $77 \pm 6\%$ (standard deviation) of DDT and $46 \pm 10\%$ of d-DDT was degraded to DDE. Deuterated DDT then is significantly more stable than DDT in 1% ethanolic sodium hydroxide. (Dachauer & Hennessy (1959) report a distinct (2×–3×) isotope rate effect in the free radical dehydrochlorination of DDT and methoxychlor.)

TDE was more difficult to dehydrochlorinate in alkali than was DDT, and the dehydrochlorinated product(s) gave poorer quantitative results by the Schechter-Haller method. No degradation could be discerned of either TDE or d-TDE after 60 minutes in 1% ethanolic sodium hydroxide. This agrees with results on TDE obtained by Müller (1946) and Cristol *et al.* (1952).

To compare absorption and degradation in living flies, 0.7 μg. of insecticide was applied to the thoraxes of 3-day-old adult house flies of R strain. This low dosage gave almost no toxic symptoms and less than 3% kill. After 24 hours, samples of 100 survivors were rinsed twice, for 10 seconds, with 25-ml. portions of reagent-grade diethyl ether to remove unabsorbed insecticide. The ether rinses were evaporated under vacuum on a rotary film evaporator, and residues were analyzed by the Schechter-Haller method. The difference between the applied dosage and the quantity of insecticide in the rinse was considered absorbed.

Degradation of absorbed insecticides was measured by pulverizing the rinsed flies with anhydrous sodium sulfate with a mortar and pestle. This brei was soaked overnight in ether, then extracted with two 25-ml. portions. The ether was filtered and then evaporated, and the residues were analyzed by the Schechter-Haller method.

The absorptions and degradations of the insecticides are shown in table 3.

Females absorbed more DDT and d-DDT than male flies. When measured by Student's "t" test, the difference between males and females was highly significant with DDT and above the 90% confidence limits with d-DDT. More DDT than d-DDT was absorbed. The difference was highly significant in males and above the 80% confidence limits in females. No dehydrochlorinated insecticide was found in the rinses.

The probability exceeds 80% that males contained more DDE than did females. In contrast to the results obtained with alkali, house flies evidently dehydrochlorinated d-DDT as readily as DDT.

More undegraded d-DDT than DDT was found inside the flies even though less d-DDT was absorbed. The un-

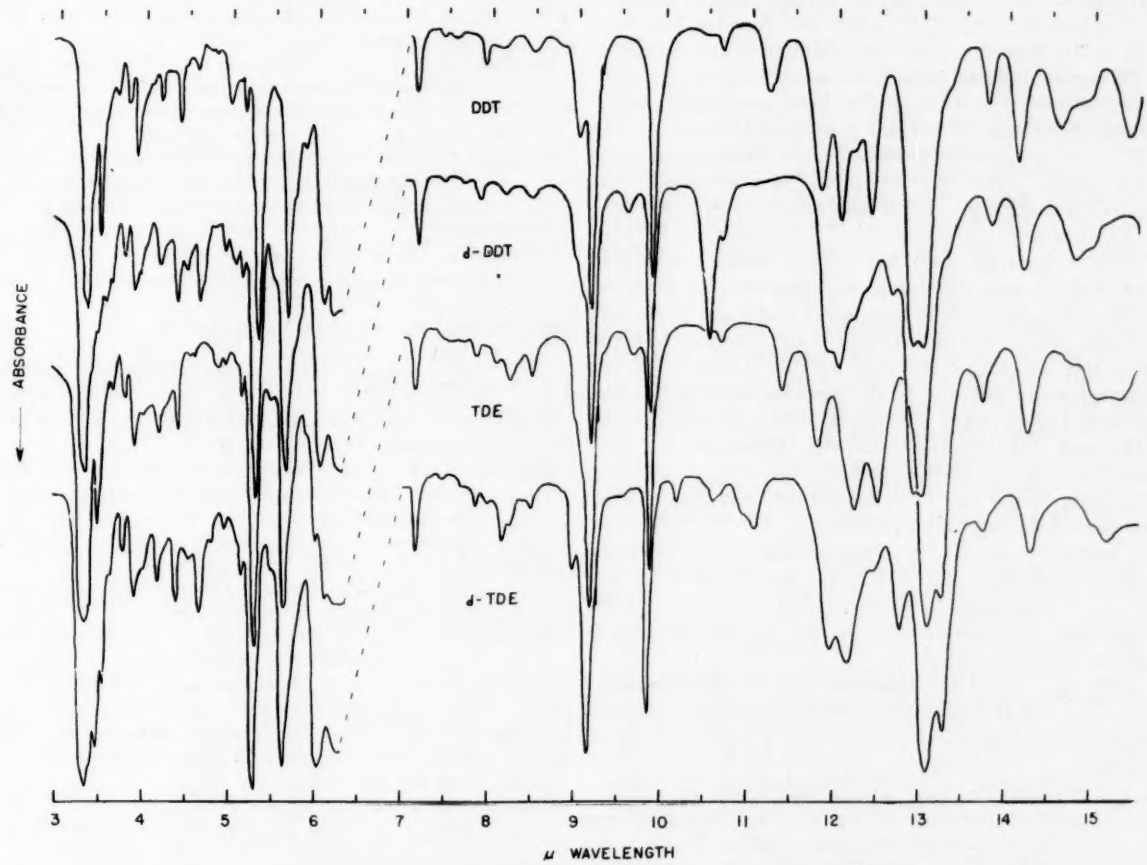

FIG. 3.—Infrared absorption spectra of DDT, d-DDT, TDE, and d-TDE. Spectra below 6.5 μ were obtained using 200 mg./ml. of carbon tetrachloride in a 3.0 mm. KBr cell; above 6.5 μ with 25 mg./ml. of carbon disulfide in 0.4 mm. KBr cell.

Table 3.—Absorption and degradation of 0.7 μg. of DDT, d-DDT, TDE, or d-TDE applied topically to R flies, then analyzed after 24 hours by the Schechter-Haller method.

Treatment	No. of Samples	Per Cent of Applied Dosage ± Standard Deviation of Individuals			
		Internal			
		Unabsorbed	Undegraded	Dehydrochlorinated	Unaccountable
DDT					
Male	12	7.6±1.5	0.1±1.3	29.3±10.9	63.0
Female	10	4.5±1.8	0 ±1.2	22.3± 9.7	73.2
d-DDT					
Male	14	9.1±2.7	2.1±1.6	29.9±11.9	58.9
Female	12	5.8±2.4	2.0±1.4	19.7± 9.1	72.5
TDE					
Male	10	4.0±2.6	6.1±4.2	8.5± 5.0	81.4
Female	14	3.3±2.7	7.9±4.5	10.6± 5.3	78.2
d-TDE					
Male	11	3.5±2.2	5.1±4.4	7.6± 5.3	82.8
Female	15	2.7±3.2	7.1±3.9	8.8± 2.8	81.4

degraded DDT does not seem to be correlated to dehydrochlorination, since the sexes did not differ in DDT content in spite of differences in DDE recovered.

The unaccountable fraction seemed unusually high and was higher in females. Although susceptible flies were not killed when held in paper cans that had been used to hold treated flies, the paper cans were analyzed. When they were rinsed with ether, ethanol, or acetone, 5.2±2.6% of the applied DDT or d-DDT was rinsed from the cans. With TDE and d-TDE 7.6±3.8% was found in rinses of the containers. Since both males and females had been held in the same container, sex differences are unknown.

Perhaps correlated with their greater loss to the containers, less of the TDE and d-TDE were found in external rinses than with DDT and d-DDT. As with the DDT analogs, females absorbed more TDE and d-TDE than males; however, the difference was within the analytical error with TDE and barely above the 50% probability level for d-TDE.

The undegraded TDE or d-TDE found inside flies exceeded the quantity found undegraded with DDT or d-DDT. No difference was found between TDE and d-TDE. Males contained less than females, but the differences are about equal to analytical error. The sex differences are not due to dehydrochlorination; females contained more of the dehydrochlorinate as well as more TDE and d-TDE. However, the differences are only near the 50% probability level. The quantity of dehydrochlorinate found for TDE or d-TDE was significantly less than the DDE found for DDT.

Conclusions.—These comparisons of deuterated analogs have suggested that DDT toxicity involves electronic structure and force fields. The absorption spectra confirmed expectations that the electron pattern remains unchanged by deuteration.

Although more DDT was absorbed, d-DDT apparently is more toxic. The differences between analogs seem inadequate to support the concept of a free-radical toxiphore.

Either house flies have an unusual facility for splitting carbon-deuterium bonds as readily as a carbon-hydrogen bond, or else removal of hydrogen is not the limiting step in dehydrochlorination of DDT and TDE.

Dehydrochlorination differs in house flies and in alkali. Since males contained more DDE than females but nevertheless more males died, this difference in toxicity cannot be related to dehydrochlorination.

Detoxication other than dehydrochlorination possibly modifies toxicity in resistant flies. Although more DDT was absorbed, and analogs were dehydrochlorinated in equal amounts, d-DDT seemed to be more toxic. The differences in slope may be due to transport differences.

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A Seasonal Study of Diapause, Reproductive Activity and Seasonal Tolerance to Insecticides in the Boll Weevil[1]

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ABSTRACT

A seasonal study of reproductive activity, incidence of diapause and seasonal tolerance to toxaphene and Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) in the boll weevil was conducted in four cotton fields in central Texas. Diapausing weevils were first found in the various fields during the period from late July through August. Once diapausing weevils were found in a field, they occurred in all subsequent collections from the field. Reproductive activity was high from June until the onset of diapause. One peak of reproductive activity occurred in September on regrowth cotton. Overwintered weevils that emerged early in the season with fat content over 10% of the dry weight were harder to kill with toxaphene than weevils of the current season collected during June and early July. Seasonal tolerance to toxaphene of up to 100-fold occurred with the onset of diapause in the populations. No evidence of seasonal tolerance to Guthion was found.

The diapausing condition in which the boll weevil, *Anthonomus grandis* Boh., survives the winter in the temperate regions of the United States was reported by Brazzel & Newsom (1959). These authors also conducted a seasonal study of the incidence of diapause in several fields and found that diapausing weevils occurred in some fields as early as the last week in July. The study reported herein was designed to determine the time of year and field conditions under which diapausing weevils occurred in central Texas. In addition, data were obtained on seasonal reproductivity of weevil populations and the time of occurrence and extent of seasonal tolerance to chlorinated-hydrocarbon insecticides.

Rainwater & Gaines (1951) demonstrated that with several chlorinated-hydrocarbon insecticides, boll weevils were progressively more difficult to control as the cotton growing season advanced. Seasonal tolerance is used in this report to describe this phenomenon and to distinguish it from insecticide resistance reported by Roussel & Clower (1957). Reiser *et al.* (1953) concluded that fat content of the boll weevil was not directly related to its resistance (tolerance) to chlorinated-hydrocarbon insecticides and that one must look for a more fundamental condition which may explain both the higher fat content of late-season weevils and the greater tolerance of these weevils to insecticides. It is possible that the various physiological changes that occur in diapausing weevils (Brazzel & Newsom 1959) may be responsible for seasonal tolerance. Dosage mortality lines were obtained for the weevil populations studied to determine if there was a correlation between seasonal tolerance and the occurrence of diapause.

MATERIALS AND METHODS.—All weevils used in this study were hand picked in the adult stage from the fruits and vegetative parts of cotton. Collections were made at weekly intervals from each field beginning when weevils were available and continuing until frost or until the cotton leafworm, *Alabama argillacea* (Hbn.), destroyed the cotton. The study included the weevil populations in four fields in the vicinity of College Station. These fields will be referred to by number and are described as follows:

Field 1, a 40-acre field on upland soil, was located 10

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miles northwest of Bryan. Collections were started during the week of May 26. The cotton was not irrigated and only one application of toxaphene was made during early July. The last collection was made the week of November 17 just prior to frost.

Field 2, a field of about 4 acres near the Entomology Laboratory, was used primarily as a source of weevils to be used in various laboratory experiments. Collections were delayed until June 16 when first generation weevils were present, to insure heavy infestations. The field was irrigated twice during July. No insecticide applications were made. The last collection was made the week of November 17 just prior to frost.

Field 3, a 12-acre field, 8 miles northeast of College Station, was located in a creek bottom. This field was not found until the week of June 30, the date of the first collection. The cotton was not irrigated and several applications of various chlorinated-hydrocarbon insecticides were made during the season. The applications were not on schedule and were improperly applied. They had little effect on the weevil population. The last collection was made the week of October 13. The following week the cotton leafworm and cattle had completely stripped the field and no weevils could be found.

Field 4, a 50-acre field located 6 miles west of College Station, was located in the Brazos River bottom. The first collection was made on May 5. The cotton was irrigated and an adequate boll-weevil-control program was followed. The last collection was made the week of October 27, after which the cotton leafworm stripped the cotton.

Sufficient numbers of weevils were collected in each sample to perform the following operations:

(a) Twenty males were dissected and classified according to age, fat condition, color of testes, diameter of testes, and number of sperm bundles.

(b) Twenty females were dissected and classified according to age, fat condition, number of mature and immature eggs in the oviducts, length of the ovaries and presence of sperm in the spermatheca and vaginal pouch.

(c) Thirty to fifty randomly selected weevils were used for determination of the lipid content.

(d) At least 120 weevils were used to obtain a dosage-mortality line.

Determination of Diapause.—The method for determination of diapause in weevils was described by Brazzel & Newsom (1959). The criteria used for determination of diapause in this study were as follows: (a) females; ovaries atrophied (not longer than 2.0 mm.), no eggs visible with 9× magnification and visible fat deposits. (b) males; testes atrophied (diameter 0.6 mm. or less), no sperm bundles present and visible fat deposits. Weevils were dissected under Ringer's solution in a wax dissecting pan with 9× magnification.

The age classifications of young and old were based on texture of the cuticle and developmental status of the reproductive organs. Weevils classified as young were less than 3 days old.

Estimates on Reproductive Activity.—Estimates on reproductive activity were obtained by counting the mature eggs found in the lateral oviducts of the dissected females.

Fat content as determined was the total ether-extractable material expressed as a percentage of the dry weight of the boll weevils. Samples of 30 or more dried and crushed weevils were refluxed for 6 hours in a micro-soxhlet apparatus as described by Brazzel *et al.* (1957).

All weevils in each collection were sexed by the snout characteristics described by Hinds & Yothers (1909). Weekly infestation records were obtained until diapause occurred in the four fields under study by counting the number of damaged squares per 100 squares in several locations. Notes were kept on the developmental status of the cotton plants when each collection was made.

Dosage-mortality lines with toxaphene and Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) were obtained for the weevils in each field when available on alternate weeks from May to October. Technical grade toxaphene and Guthion were serially diluted in acetone to obtain the various dosages. One μl of insecticide was applied topically to the metathoracic region with a microapplicator similar to the device described by Trevan (1922) and modified by Roan & Maeda (1953). The LD 50's were computed by probit analysis on an IBM processing machine. The LD 50's were expressed in terms of milligrams of toxicant per gram of body weight.

RESULTS OF SEASONAL STUDY.—The results obtained from the four fields included in the study are given in table 1. The reproductive activity, fat content, and percentage diapausing weevils for the population in Field No. 1 are presented graphically in figure 1.

The first collection was made May 5 in Field 4 when the cotton had two true leaves. Squares were not present on the cotton when the second collection was made May 12. The LD 50 for toxaphene during this 2-week period was from 4 to more than 5 mg./gm. weevil weight and the fat content was more than 20%. When the third collection was made on May 19 a few tiny squares were found and the fat content was 17.1%. The LD-50 for Guthion in this collection and the one of the prior week was 0.0011 and 0.0033 mg./gm. weevil weight, respectively.

On May 26 collections were made in Fields No. 1 and No. 4. In both fields the weevils were feeding on the small squares but no eggs were found in a sample of 100 squares that were dissected. Mature eggs were found in the females as early as May 12 and by May 26 an average of 1.4 mature eggs per female was found in the weevils that were dissected. The fat content was below 10% in the weevils collected in both fields at this time.

Weevils had started ovipositing in squares by June 2. First-generation weevils were found in Fields 2 and 4 on June 16 and in Field 1 on June 23. Reproductive activity had reached 5.2 eggs per female in both Fields 1 and 4 immediately prior to the emergence of the first generation. Dilution of the overwintered population by the young weevils lowered the eggs-per-female during the following 2-week period to from one to two eggs per female. The overwintered females dissected in these collections contained most of the eggs recorded.

During the week prior to emergence of second generation weevils four to more than six eggs per female were found in the oviducts of weevils from Fields 1, 2, and 3. Field 4 did not follow the same pattern because of the fact that an insecticide program was in effect. The collection in Field 4 on June 30 was made on the third day after

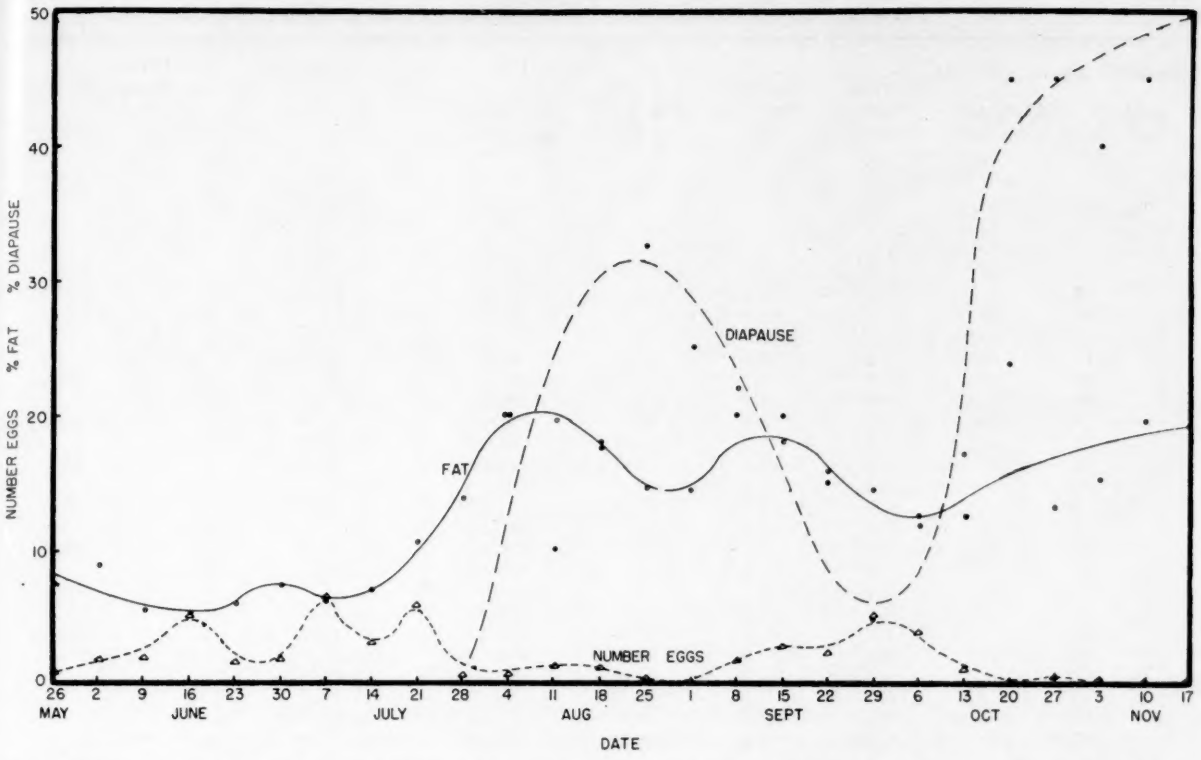

FIG. 1.—Seasonal record of reproductive activity, fat content and diapausing boll weevils in dry-land untreated cotton, Field No. 1.

an application of endrin. No mature eggs were found in the oviducts and 59.4% of the weevils were considered young (table 1). Thereafter, large numbers of eggs per female were found in the collections from Field 4 in which a low percentage of the weevils were young until diapausing weevils were found on August 25. The emergence of second-generation weevils in Fields 1, 2, and 3 during the first half of July resulted in few eggs per female. These data indicate that the emergence of first- and second-generation weevils in untreated fields may be determined by the reproductive activity of the females in the population.

During the period from the start of fruiting in early June until mid-July the cotton was growing and fruiting vigorously, and weevil-punctured squares ranged up to about 70%. Fat content of the weevil populations was less than 10% of the dry weight. During the period of vigorous plant growth, low fat content, and high reproductive activity the boll weevils were quite susceptible to toxaphene. The LD-50 ranged from 0.1 to 0.6 mg./gm. body weight.

During the latter part of July the cotton in Fields 1, 2, and 3 showed signs of water stress, reduced vegetative growth, reduced fruiting and shedding of fruit. With the reduced numbers of squares and increasing numbers of weevils, the square infestation increased rapidly to approximately 100%, at which time the weevils began to attack small bolls. By the last week of July the fat content of the weevils in these three fields was more than 10% and diapausing weevils were found in Field 3. Diapausing weevils were found in Fields 1 and 2 during the first week of August. With the onset of diapause and increased fat content of the weevil population, there was an immediate and large increase in the amount of toxaphene required to kill 50% of the weevil population.

Fruiting activity of the cotton was prolonged in Field 4 by irrigation. Diapausing weevils were first found during the last week of August. Susceptibility of the weevils in this field was rather erratic during August, but there was a considerable decrease in susceptibility during the course of the month as the fat content of the weevils increased. After the occurrence of diapause in the population, the tolerance to toxaphene followed the same pattern as in the other fields.

With the exception of three tests conducted during May and June on weevils from Field 4, all the toxicity data for Guthion were obtained during September, October, and November. These results show no indication of seasonal tolerance in the boll weevil to Guthion.

Reproductive activity of the weevil populations in Fields 1, 2, and 3 was high during June and early July except when a new generation of weevils emerged. During the latter part of July and early August, when diapause occurred, there was a rapid drop in egg production.

Lack of moisture caused complete cessation of growth and fruiting during August in Fields 1, 2 and 3. Cotton began to open early in the month and no squares were available for food during the latter part of the month. This period of food stress resulted in lower fat content of the weevil populations. Undoubtedly, some migration from the fields occurred during this time, but sufficient weevils were present at all times to obtain samples readily.

Rains during the latter part of August and September

Table 1.—Seasonal record for the boll weevil population in dry-land and irrigated cotton.

Date 1958	Punctured Squares	Mature Eggs/ Female	Young Weevils (%)	Diapause (%)	Sex Ratio M:F	Fat (%)	LD-50 Mg./Gm. Weevil Weight	
							Toxaphene	Guthion
				Field No. 1				
May 26	—	0.8	—	—	67:70	7.4	32.73	
June 2	—	1.6	—	—	57:50	8.8	.10	
9	—	1.8	—	—	119:93	5.4	.38	
16	—	5.2	—	—	44:53	5.2		
23	—	1.5	29.2	—	28:26	5.9		
30	60	1.7	20.0	—	36:24	7.3	.34	
July 7	50	6.5	5.0	—	46:29	6.3		
14	68	2.9	10.0	—	94:79	7.0	.35	
21	80	5.9	5.0	—	60:39	10.5		
28	97	0.6	15.0	—	98:78	13.8	32.76	
Aug. 4	99	0.7	7.5	20.0	120:68	20.0		0.0012
11	—	1.3	7.5	10.0	241:120	19.5	21.38	
18	—	1.2	10.0	17.5	218:123	17.8		.0062
25	—	0.4	7.5	32.5	226:125	14.5	304.10	
Sept. 1	—	0.05	2.5	25.0	377:196	14.4		.0019
8	—	1.8	7.5	20.0	267:118	22.1	9.94	
15	—	2.9	7.5	20.0	178:62	18.0		.0019
22	—	2.3	2.5	15.0	159:93	15.9	2.32	
29	—	5.2	2.5	5.0	136:62	14.5		.0021
Oct. 6	—	3.8	15.0	12.5	163:98	11.7	19.15	
13	—	1.2	20.0	12.5	161:121	17.1		.0024
20	—	0.0	20.0	45.0	164:102	23.8	17.06	
27	—	0.6	15.0	45.0	72:47	13.2		
Nov. 3	—	0.3	7.5	40.0	70:67	15.2		
10	—	0.0	17.5	45.0	57:50	19.6		
17	—	0.05	5.0	50.0	64:57	19.1		
				Field No. 2				
June 16	100	0.5	12.9	—	36:26	6.2		
23	80	0.4	47.5	—	42:25	6.5		
30	63	2.9	17.5	—	51:30	6.8		
July 7	80	4.2	12.5	—	55:28	5.0		
14	73	4.4	20.0	—	116:80	6.7	0.50	
21	79	0.7	27.5	—	59:37	7.2		
28	98	2.0	17.5	—	145:83	12.6	1.06	
Aug. 4	97	0.7	25.7	5.7	131:46	18.7		
11	—	0.6	40.0	7.5	145:70	17.5	20.35	
18	—	1.0	22.5	17.5	117:100	17.6		
25	—	0.7	7.5	7.5	241:111	12.4	4.09	
Sept. 1	—	1.1	5.0	30.0	226:105	14.3		
8	—	2.2	7.5	20.0	275:186	14.5	3.16	
15	—	0.3	25.0	22.5	146:80	15.1		
22	—	0.7	10.0	32.5	180:116	20.0	2.36	
29	—	0.4	7.5	40.0	164:67	24.6		0.0029
Oct. 6	—	0.3	17.5	32.5	154:72	16.4	2.44	
13	—	0.0	17.5	40.0	114:76	20.9		.0030
20	—	0.2	12.5	47.5	186:167	19.3	13.51	
27	—	0.5	2.5	62.5	62:40	19.2		
Nov. 3	—	1.6	7.5	27.5	57:41	13.8		
10	—	0.8	5.0	47.5	53:51	18.1		
17	—	1.4	0.0	35.0	55:48	15.1	5.98	.0020
				Field No. 3				
June 30	95	5.3	18.2	—	27:23	6.3		
July 7	80	2.2	16.7	—	6:6	—		
14	68	3.5	15.0	—	48:57	2.7		
21	65	2.0	12.5	—	56:43	6.2		
28	85	1.2	17.5	7.5	107:68	15.9	0.38	
Aug. 4	—	1.1	20.0	7.5	63:44	21.8		
11	—	0.6	32.5	25.0	135:91	15.6	10.94	
18	—	0.2	12.5	45.0	153:91	23.2		0.0027
25	—	0.2	17.5	47.5	220:161	13.0	2.79	
Sept. 1	—	0.6	10.0	40.0	153:78	23.0		.0025
8	—	0.4	5.0	62.5	286:216	21.9	30.67	
15	—	1.3	2.5	42.5	230:119	24.7		
22	—	1.9	10.0	37.5	154:74	22.9	18.98	
29	—	2.3	2.5	50.0	67:30	21.2		.0018
Oct. 6	—	1.5	26.3	20.0	101:83	24.7	3.68	
13	—	0.1	17.5	40.0	130:76	23.0		.0022

Table 1.—(Continued)

DATE 1958	PUNCTURED SQUARES	MATURE EGGS/ FEMALE	YOUNG WEEVILS (%)	DIAPAUSE (%)	SEX RATIO M:F	FAT (%)	LD-50 MG./GM. WEEVIL WEIGHT	
							Toxaphene	Guthion
				Field No. 4				
May 5	—	—	—	—	21:18	—	4.00	
12	—	0.3	—	—	20:16	25.4	5.17	0.0033
19	—	0.1	—	—	58:70	17.1		.0011
26	—	1.4	—	—	44:37	9.5		
June 2	—	1.7	—	—	63:37	3.3		
9	—	5.2	—	—	132:123	7.4	.68	.0015
16	—	1.3	37.5	—	23:41	7.0		
23	—	1.0	57.7	—	18:41	7.0		
30	40	0.0	59.4	—	31:32	11.0	.14	
July 7	14	3.0	5.0	—	56:45	3.4		
14	37	3.8	11.8	—	77:68	3.3	.35	
21	78	0.8	75.0	—	106:92	7.0		
28	40	0.8	60.0	—	37:43	7.6		
Aug. 4	85	5.4	12.5	—	122:94	8.9	3.03	
11	95	1.6	35.0	—	148:125	13.1	.27	
18	100	2.4	27.5	—	195:131	16.5	11.90	
25	100	0.1	30.0	7.5	279:208	8.4	2.00	
Sept. 1	—	0.5	17.5	17.5	191:109	20.2		.0020
8	—	0.5	17.5	25.0	219:130	15.9	32.93	
15	—	0.3	32.5	17.5	132:63	18.4		.0023
22	—	1.2	5.0	25.0	124:69	16.2	3.99	
29	—	0.5	7.5	55.0	171:132	18.3		.0036
Oct. 6	—	0.2	2.5	55.0	204:105	24.6	82.26	
13	—	0.3	32.5	30.0	119:77	19.6		.0028
20	—	0.05	22.5	40.0	128:92	12.1	21.22	
27	—	0.0	12.5	52.9	17:17	—		

resulted in vigorous regrowth of the cotton in all the fields. Small squares were present by early September and the cotton continued to fruit throughout the month. An increase in reproductive activity in the boll weevil populations coincided with the regrowth and fruiting of the cotton plants. Generally, as the reproductive activity of the populations increased during September, the percentage of diapausing weevils decreased.

The first cool weather of the season began during the last week of September when the minimum temperature was below 60° F. from September 29 through October 4. During the next 2 weeks the reproductive activity dropped to extremely low levels and remained low for the remainder of the season. Also, there was an increase in the incidence of diapause in the populations following the cool weather.

The sex ratio of the weevils followed the same general pattern in the three untreated upland fields. Early in the season and until about the time of emergence of second-generation weevils there were a few more males than females. During the remainder of the season, with a few exceptions, there were approximately two males to each female. This increased proportion of males was particularly pronounced during the onset of diapause and for several weeks thereafter. Observations indicate that the change in sex ratio of the population is related to increased activity of the reproducing females in search of suitable oviposition sites. It appeared that as square infestations reached 70% to 100% many females migrated from the field in search of undamaged squares for oviposition sites.

DISCUSSION.—The results of the toxicity tests indicated low susceptibility to toxaphene of overwintered boll weevils collected early in the spring while the fat content was above 10%. However, after cotton started squaring, the fat content dropped below 10% rapidly and the weevils were highly susceptible to toxaphene during June and early July. With the onset of diapause in late July and August the fat content of the population increased, reproductive activity decreased and there was an increase in tolerance to toxaphene which amounted to as much as 100-fold within 2 weeks. This phenomenon did not occur at the same time of the season in all fields, indicating that it resulted from some interaction of the insect-host plant relationship. These results indicate that the occurrence of seasonal tolerance to chlorinated-hydrocarbon insecticides is a result of the field conditions in each particular field.

Several tests were conducted with dieldrin and endrin during the latter part of the season. The results indicated that there was a seasonal tolerance to these materials just as in the case of toxaphene. Apparently seasonal tolerance in the boll weevil is common to the chlorinated-hydrocarbon insecticides.

Results obtained in this study indicate that diapausing weevils occur in the fields of central Texas sometime during a period from the latter part of July to the first of September. If at any time during this period the recommended control measures are not followed and the weevil population reaches the point that 70% to 100% of the squares are infested and bolls are attacked, difficulty would be expected in obtaining control with a chlorinated-hydrocarbon insecticide because of seasonal tolerance. However, if the weevils are not resistant to chlorinated-hydrocarbon insecticides and the recommended control practices are followed, these materials

will give satisfactory control throughout the season.

Reproductive activity of weevils in untreated fields was high during June and most of July. There were three peaks of reproduction (fig. 1). The first occurred just prior to emergence of the first generation weevils, the second just prior to emergence of the second generation and the third about a week before the occurrence of diapausing weevils in the population. Egg production of older weevils was high throughout this period but the dilution of the population by large numbers of young weevils of the first and second generations lowered the average number of eggs per female. However, after the onset of diapause in the population very few eggs were found in any of the weevils.

During late August and September there was adequate rainfall to cause regrowth and fruiting of the cotton plants. This resulted in another peak of reproductive activity in the nondiapausing portion of the population. This increase in egg production was terminated by cool weather during the last week of September. Although adequate food was present in all four fields, the reproductive activity of the populations remained low after this first cool weather.

These results indicate that reproductive activity of the boll weevil is intimately associated with the physiological state of the cotton plant. Vigorous plant growth and fruiting resulted in increased reproductive activity, even as late as September.

Once diapausing weevils were found in a field, they were found in all subsequent collections from that field. However, a lower percentage of diapausing individuals was present during September, as reproductive activity increased, than earlier in the season (fig. 1). After the occurrence of diapause, the fat content of the populations remained relatively high with the exception of slight reductions during the period of food stress in August and increased reproductive activity during September.

With the occurrence of minimum temperature below 60° F. there was a marked reduction in reproductive activity, increase in fat content and increase in the incidence of diapause. Observations indicated that after cool weather began considerable change in weevil behavior occurred. Since reproduction was almost at a standstill, the weevils were engaged principally in feeding. Feeding activity seemed to be limited to relatively few squares in which many feeding punctures were found. Apparently, very little movement from fruit to fruit occurred, as many unpunctured squares and blooms were present and large populations of weevils were present in all fields. During this period many unmated females were present in the populations. This condition could have been caused by the reduced activity of the weevils during the late fall after onset of cool weather.

It is not known how late in the season diapausing weevils emerged from winter quarters, but overwintered weevils were found in collections made as late as July 21. Diapausing weevils of the current season were found in the same field on July 28. These results support the statement by Brazzel & Newsom (1959) that it appears certain that boll weevils in diapause are present in woods throughout the year. Also, in untreated fields, weevils that diapaused during the previous season are present at the same time weevils of the current season are going into diapause.

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The Fate of Phorate in Soils[1]

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Abstract

Studies were conducted to determine the hydrolysis, oxidation, insecticidal efficiency, persistence, and the degree of binding of phorate, formerly designated as Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate) in three soils and quartz sand.

Phorate applied as a soil treatment in the field was more available to cabbage and potatoes grown in a sandy soil than in a clay-loam soil as indicated by insect-control data and anticholinesterase assay. A measurement of P^{32}-labeled phorate uptake by peas from three soils and quartz sand showed that the largest amounts of toxicant were taken from quartz sand and followed by lesser amounts from a sandy soil, clay loam, and muck, in that order.

Extraction, column chromatographic and partitioning techniques showed that soil applications of phorate are partially oxidized, hydrolyzed, and bound to the soil. Chloroform extractions of radioactivity from the soils at 7, 14, and 28 days after treatment were identified as phorate plus a mixture of the phosphorodithioate sulfoxide and the phosphorodithioate sulfone of phorate. Only a very small amount of the radioactivity extracted from the soils could be identified as hydrolysis products. A large portion of the radioactivity remained bound to the soil and could not be identified. Radioactive phorate added to quartz sand was rapidly hydrolyzed, but no oxidation products were detected.

A study of phorate volatilization from the soils showed that within an hour after treatment the sandy soil, silt loam, and muck had lost respectively, 25%, 20%, and 18% of the radioactivity applied. However, after this initial loss, very little or no volatilization occurred. Phorate was lost rapidly from quartz sand and a steel surface with less than 10% of the radioactivity remaining 24 hours after treatment.

The method of applying phorate, formerly designated as Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate), to the soil for subsequent translocation to plant foliage is of considerable interest to economic entomologists at present. Reports of phorate soil treatments indicate varying degrees of control of insects infesting the foliage of plants grown in the treated soils. Dobson & Watts (1957) failed to control spotted alfalfa aphids with granulated furrow treatments of phorate at 1 pound per acre applied at seeding time. Granulated treatments applied at planting time gave significant control of aphids on cotton for 5 months, but did not reduce cotton boll weevil, *Anthonomus grandis* Boh., populations (Hopkins *et al.* 1958). At the same time they noted that varying amounts of phosphate fertilizer did not affect phorate absorption by cotton plants as indicated by cotton aphid mortality. Phorate granules applied at 1, 2, and 4 pounds per acre to the seed furrow of turnips gave effective aphid control through harvest time (Wilcox & Howland 1957). Granulated furrow treatments gave higher turnip aphid, *Rhopalosiphum pseudobrassicae* (Davis), control than phorate applied to the furrow as sprays. In general, reports of phorate soil treatments indicate a lasting residual effectiveness.

This investigation deals with the fate of phorate in three soil types and quartz sand. Studies were conducted to determine the hydrolysis, oxidation, insecticidal efficiency, persistence, and the degree of binding of phorate in the different soils and sand.

Materials and Methods.—*Field Experiments.*—Field tests were conducted with potatoes and cabbage to measure the uptake of phorate from two soil types, a sandy soil (Plainfield sand) near Rhinelander, Wisconsin, and a silt loam (Clyde silt loam) near Kenosha, Wisconsin. The comparative uptake of phorate from the two soils was measured by anticholinesterase analysis and insect control data.

Soil broadcast treatments of granulated phorate at 2 and 8 pounds per acre and emulsifiable phorate at 8 pounds per acre were applied to potato plots 20 feet square and replicated three times in a randomized complete block design at both locations. After the insecticides were applied with a knapsack sprayer and a granular distributor, the soils were disked to a depth of 3 inches and planted to Sebago potatoes. Foliage samples (200 grams) were taken at 1, 2, and 3 months after planting and frozen for subsequent anticholinesterase assay.

Cabbage transplant water treatments with a phorate emulsion at a rate of 2 and 6 pounds per acre were replicated four times at both locations. Imported cabbageworm, *Pieris rapae* (L.), and cabbage looper, *Trichoplusia ni* (Hübner), counts were made at both locations at 1 and 2 months after transplanting by counting the number of each insect found per 10 plants in each of the 4 replicates. Cabbage aphid, *Brevicoryne brassicae* (L.), counts were also taken at Kenosha by counting the number of plants containing aphid colonies per plot. Plant samples (12 plants per treatment) taken at 2, 4, and 8 weeks after transplanting were frozen for later anticholinesterase assay.

The anticholinesterase method used was a modification of that described by Hensel *et al.* (1951). A homogenate of the potato or cabbage tissue was prepared by macerating 100 gm. of frozen tissue with an equal weight of water in a Waring Blendor and straining the brei through four thicknesses of cheesecloth. After adjusting the liquid to pH 8.0 various aliquots of the homogenate (0.1, 0.3, 1.0, 3.0, and 6.0 ml.) were added to 3 ml. of whole human blood at pH 8.0 in centrifuge tubes and brought to a total volume of 10 ml. with distilled water. After incubation at 37.5° C. for 60 minutes, 1 ml. from each tube was added to 1 ml. of a phosphate buffer (pH 8.0) in small beakers. Following the initial pH readings, 0.2 ml. of acetylcholine was added to each beaker, after which the samples were

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incubated at 37.5° C. for 2 hours before taking the final pH readings.

Uptake of P^{32}-Labelled Phorate by Peas from Three Soils and Quartz Sand.—Radioactive phorate (preparation described by Bowman (1958)) was utilized in greenhouse and laboratory studies. The effect of different soil types upon the uptake and persistence of phorate was studied following a drench application of the P^{32}-labeled toxicant to three soils and quartz sand. Peas (Perfection variety) were grown in the various soil materials contained in cans ($4\frac{1}{2}''$ high$\times 2\frac{1}{2}''$ dia.). The soils were treated with 2 mg. of phorate in 15 ml. of water when the plants (three per can) were 2 to 3 inches tall. At the time of application the top inch of soil in the cans contained less than 10% free water. Plant samples (three replicates per treatment) were cut and frozen for extraction at 4, 8, 16, and 24 days after treatment. The frozen samples were macerated with a mortar and pestle in four times their weight of distilled water. Five ml. of this brei were partitioned with an equal volume of chloroform. The chloroform layer contained the phorate plus its oxidation products and the aqueous layer contained the nontoxic hydrolysis products. The concentration of radioactivity found in each layer and in dried plant residue was recorded.

The rate of insecticide leaching through the various materials was also measured. After the plant samples were removed the soil from each can was divided into three equal $1\frac{1}{2}$ inch layers, air dried for 24 hours and mixed, after which, 1-gram samples from each layer were counted for radioactivity. Samples from the quartz sand were counted prior to drying because of the great volatilization of phorate which was known to occur from the dry surface of the sand. The hydrolysis rate of phorate in quartz sand was measured by extracting and partitioning the radioactive materials from 5 gm. of the sand with 5 ml. of water and 5 ml. of chloroform at the time when plant samples were removed. The hydrolysis of phorate in the agricultural soils was determined only after the 24-day plant samples were removed.

Oxidation and Hydrolysis of Phorate in Soils and Sand.—The oxidation and hydrolysis of phorate in a sandy soil, a silt loam, a muck, and quartz sand were determined by extraction and column chromatographic procedures. The soils and sand were treated with P^{32}-labeled phorate contained in 5 ml. of chloroform at a rate of 1.45 mg. of phorate per 300 cubic cm. (approx. 3 lbs. per acre) of soil. After adding the phorate the material was mixed thoroughly, voided of chloroform, and mixed again. Each soil treatment was then divided into 10 equal portions and placed in shell vials (22 mm.$\times$95 mm.). Enough water was added to each vial of soil to obtain a condition approximately equivalent to field capacity. The vials were then placed in a humidity chamber held at 100% humidity and 23° to 25° C. for the duration of the experiment. Three vials or replicates of each soil were removed at 7, 14, and 28 days after treatment for analysis. The tests with quartz sand were carried out at a different time and vials were removed at 8, 16, and 32 days after treatment. Only one replicate of each soil was utilized for the zero-time interval. Each of the three soil replicates was removed from the vials and allowed to air dry for 24 hours. The samples were then extracted with 200 to 300 ml. of chloroform in a Waring Blendor, and washed on a Büchner funnel. Radioactivity counts in the chloroform extract and in the soil residues were recorded. The soil samples were then extracted a second time with 100 ml. of water in a Waring Blendor. After filtering through a Büchner funnel, 5 ml. of the water extract was partitioned with 5 ml. of chloroform and counted. Total amounts of radioactivity in chloroform extracts, in aqueous extracts, and as bound forms to the soils were calculated. Adjustments were made for the differences in self absorption among the different soils.

The radioactive materials from the chloroform extracts were identified by the use of column chromatographic procedures and partitioning properties based on the techniques used by Bowman & Casida (1957). The chloroform-soluble materials from the three replicates were combined for column separation. The organophosphorus peaks were detected solely by radioassay. The partitioning properties of the various peaks were determined with hexane, acetone and water in 2.5:2.0:3.5 volume ratios.

Volatilization of Phorate from Soil Surfaces.—The volatilization of phorate from a sandy soil, a silt loam, a muck, quartz sand, and a metal surface was studied with the aid of radioactive tracers. Two cubic centimeters of the dry soil material contained in a nickel coated planchet (7 mm. deep$\times$24 mm. in dia.) were surface sprayed with a phorate emulsion contained in a hand atomizer. The P^{32}-labeled phorate spray contained 10% acetone, 0.05% Triton X-155, and 90% water, plus the toxicant. A plastic jacket was placed around and over the side of each planchet to prevent any contamination of the metal container itself. Each planchet contained 26.6 ($\pm$3.2) micrograms of phorate in approximately 0.015 ml. of water after treatment. An empty nickel-coated planchet was treated in a manner similar to that described for planchets containing soil or sand. All treatments were replicated four times with radioassay counts being taken at 0, 53, and 160 minutes, and $\frac{1}{2}$, 1, 3, and 9 days after phorate application. During the various interims the planchets were held under normal room temperatures and humidity.

The soils utilized in these studies were analyzed by the Soils Department, University of Wisconsin, for mechanical composition, organic matter content, base exchange capacity, pH, and pounds of available nitrogen, potassium and phosphorus per acre. The soils and their various properties are given in a previous publication (Getzin & Chapman 1959).

RESULTS AND DISCUSSION.—*Field Experiments.*—The comparative uptake of phorate from soil applications to a sandy soil and silt loam by potatoes as indicated by anticholinesterase assay is shown in table 1. The data are presented as the reciprocal of the amount of tissue required to give 50% inhibition of cholinesterase. Tissue from potatoes grown in the treated Plainfield sand was able to cause a much greater inhibition of cholinesterase than tissue from potatoes grown in the Clyde silt loam. At harvest time (90 days after planting) plants grown in the sandy soil were effective esterase inhibitors even at the low rate of 2 pounds of granulated phorate per acre. Plants from soils treated with the applications of phorate in granulated and emulsion form at 8 pounds per acre in the Clyde silt loam gave only a small amount of esterase inhibition at 30 days after planting. Plant samples

Table 1.—Comparative uptake of phorate from two soils by potatoes as indicated by anticholinesterase assay.

Phorate Treatment (Soil Broadcast)	Anticholinesterase Agent Residues Present at Indicated Days After Treatment[a]		
	30	60	90
	Plainfield sand		
Granules 2 lb./A.	1.0	0.5	0.3
Granules 8 lb./A.	2.7	1.9	1.1
Emulsion 8 lb./A.	2.8	1.1	0.6
	Clyde silt loam		
Granules 2 lb./A.	<0.1	<0.1	<0.1
Granules 8 lb./A.	0.2	<0.1	<0.1
Emulsion 8 lb./A.	0.3	<0.1	<0.1

[a] Values expressed as the reciprocal of the amount of tissue required to give 50% inhibition of cholinesterase.

taken 60 and 90 days after treatment gave small amounts of inhibition on the order of 5% to 10% when using 3 grams of tissue, thus indicating the presence of phorate antiesterases, but in very small amounts. Because of low and erratic insect populations no insect control data are presented. However, in other experiments (Knoke 1959) insect control has been obtained up to harvest time with similar soil treatments. There was a slight indication that the granulated treatment was more effective than the emulsifiable treatment after the first 30 days in the Plainfield sand. Analysis of tuber samples taken at harvest time gave no indication of any cholinesterase inhibition resulting from a phorate-derived antiesterase.

Cholinesterase assay of cabbage treated with transplant water applications of phorate in the sandy soil and silt loam soil also indicated more effective insecticide uptake from sandy soils (table 2). At 15 days after treatment a high degree of esterase inhibition resulted from tissue samples of cabbage treated with 2 and 6 pounds of phorate per acre in both soil types. At harvest time (60 days after transplanting) antiesterases were still present in rather high amounts in cabbage grown in the sand. Cabbage treated with 6 pounds of phorate per acre in the silt loam also caused a fair amount of inhibition, while cabbage treated with phorate at 2 pounds per acre in the loam inhibited cholinesterase only slightly.

Higher insect control was obtained from the Plainfield sand treatments than from the Clyde silt loam treatments (table 3). Good control of imported cabbageworms

Table 2.—Comparative uptake of phorate from two soils by cabbage as indicated by anticholinesterase assay.

Phorate Dosage (Transplant Water)	Anticholinesterase Agent Residues Present at Indicated Days After Treatment[a]		
	15	30	60
	Plainfield sand		
6 lb./A.	28.6	15.4	5.4
2 lb./A.	25.3	7.1	0.8
	Clyde silt loam		
6 lb./A.	26.0	7.5	1.1
2 lb./A.	10.0	2.2	0.2

[a] Values expressed as the reciprocal of the amount of tissue required to give 50% inhibition of cholinesterase.

Table 3.—Control of cabbage insects by phorate transplant water treatments in two soils.

Soil	Actual Phorate (Lb./A.)	Per Cent Control (Days After Treatment)	
		30	60
		Imported cabbageworm	
Plainfield sand	6	99	—
Clyde silt loam	6	96	32
Plainfield sand	2	97	88
Clyde silt loam	2	56	32
Plainfield sand[a]	Control	34	21
Clyde silt loam[a]	Control	42	13
		Cabbage looper	
Plainfield sand	6	91	—
Clyde silt loam	6	62	0
Plainfield sand	2	64	56
Clyde silt loam	2	0	0
Plainfield sand[a]	Control	46	39
Clyde silt loam[a]	Control	9.5	26
		Cabbage aphid	
Clyde silt loam	6	100	—
Clyde silt loam	2	100	—
Clyde silt loam[b]	Control	5	

[a] Insects per 10 plants.
[b] Colonies per 25 plants.

resulted from both phorate applications in the sandy soil and at the high rate (6 pounds per acre) in the silt loam 30 days after treatment. At 2 pounds per acre poor worm control was obtained in the heavy soil. Only the treatment in the sandy soil gave reasonable control 60 days after application. Overall cabbage looper kill was poor with the exception of the high phorate rate on the sandy soil. No looper control resulted from the 2-pound application in the silt loam at either time interval. Similar results were obtained at harvest time with the high insecticide rate. Excellent cabbage aphid control was obtained when aphids colonized the cabbage grown in the silt loam at 30 days after transplanting. No aphid population occurred on the plots in the heavy soil toward the end of the season or in the sandy soil area at any time.

A combination of the phorate plus its solvent and emulsifier caused varied degrees of phytotoxicity at both rates in both soils. The high rate of toxicant in the sandy soil killed many of the plants so that very few were available 60 days after treatment. Although plants were initially stunted from the treatments in the silt loam, no damage was evident at harvest time.

Uptake of P^{32}-Labeled Phorate by Peas from Three Soils and Quartz Sand.—Phorate applied to soils and quartz sand by a drench method was more effectively absorbed by peas from the quartz sand and sandy soil than by plants from the silt loam or muck (table 4). The highest concentration of toxic materials (chloroform-soluble components) in peas occurred at 8 days after treatment. The sharp drop (after 16 days) in the chloroform-soluble radioactive materials of peas grown in quartz sand probably resulted from hydrolysis of the phorate in the sand medium. Chloroform and water partitions of the radioactivity extracted from the quartz sand showed that 43%, 45%, and 61% hydrolysis of phorate had occurred at 8, 16, and 24 days, respectively, after treatment. The amount of radioactivity remaining in the tissue residue increased over a period of 24 days with all treatments.

Table 4.—Uptake of P^{32}-labeled phorate from three soils and quartz sand by peas.

Soil	Total p.p.m. of Extractable P^{32}-Labeled Compounds (Days After Treatment)				Per Cent of Extractable Radioactivity as Toxic Components (Days After Treatment)				Per Cent of Total Radioactivity Bound in Tissue Residue (Days After Treatment)			
	4	8	16	24	4	8	16	24	4	8	16	24
Quartz sand	94	149	150	90	71	64	46	31	11	14	19	25
Plainfield sand	28	60	82	81	60	59	39	39	8	8	12	21
Clyde silt loam	23	30	23	21	62	43	38	18	9	7	13	29
Muck	7	8	5	10	45	28	10	17	7	6	9	29

The amount of leaching from the top 1½ inches of each material after various intervals is shown in figure 1. The soils and sand were watered every 2 days with 1 to 3 cm. of water depending upon the moisture conditions, with a total of 25 cm. in depth being applied by the end of the experiment, 24 days after treatment. The radioactive materials leached rapidly in the quartz sand and Plainfield sand. Very little leaching occurred from the top 1½ inches of the silt loam and muck. After 25 cm. of water had been applied to the soils and sand, during the 24-day period, the top 1½ inches of quartz sand, Plainfield sand, Clyde silt loam, and muck contained 23%, 48%, 87%, and 93%, respectively, of the total radioactivity in the cans.

Oxidation and Hydrolysis of Phorate in Soils and Sand.—The fate of phorate applied to three agricultural soils and quartz sand at various intervals after treatment is shown in table 5. Extraction, column chromatographic and partitioning techniques showed that phorate is partially oxidized, hydrolyzed and bound to the soil. In general, after a period of 4 weeks the percentage of phorate decreased while the oxidation products and/or the bound radioactive materials increased. A comparison of chloroform extractable radioactive compounds showed that higher percentages of oxidation products were formed in the Plainfield sand than in either of the other two soils. Initiation of phorate oxidation occurred rapidly as shown by the amount of oxidative materials present in zero-day samples from all soils. In this experiment only a small percentage of the extractable radioactivity was identified as hydrolysis products of phorate. Rather large amounts of radioactivity remained bound to the soil after the two extractions. The Plainfield sand bound the least amount of radioactivity while the greatest amount was bound in the muck soil. It has been shown that organic constituents play an important part in the binding of Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical) (Getzin & Chapman 1959). No doubt organic constituents of soil are equally important in the case of phorate and its derivatives.

The chloroform extractable materials placed on the Celite columns were eluted in only two detectable peaks regardless of the soil or the time interval after treatment. Based on the results of Bowman & Casida (1957), the first peak was identified as phorate by the position of elution and by its partitioning properties in hexane, acetone, and water. The second peak was identified as a mixture of the phosphorodithioate sulfoxide and the phosphorodithioate sulfone of phorate. The second peak from the Plainfield sand at 28 days after treatment could not be identified as either the dithioate sulfoxide, the sulfone, or as a mixture of the two by its partitioning properties, but

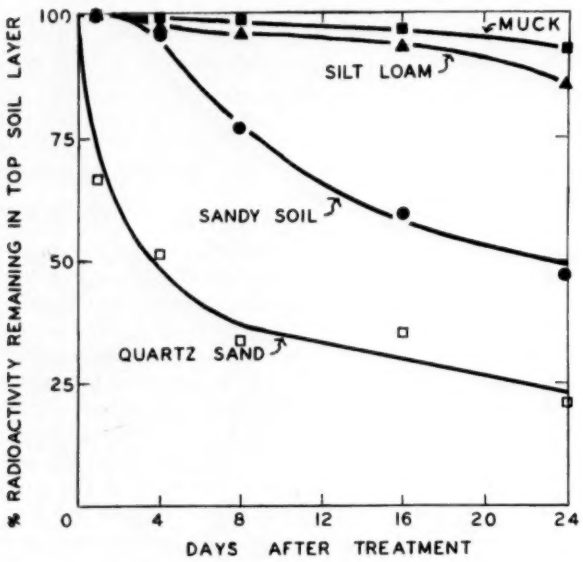

Fig. 1.—The amount of phorate leached from a 1½ inch soil layer by normal watering procedures for pea plants.

Table 5.—The fate of phorate applied to three soils and quartz sand at different intervals after treatment.

Days After Treatment	Per Cent of Radioactivity Detected as: Phorate	Oxidation Products	Hydrolysis Products	Bound to Soil
	Plainfield sand			
0	75	11	0	14
7	24	51	2	23
14	20	54	2	24
28	12	61	2	25
	Clyde clay loam			
0	67	13	0	20
7	22	33	5	40
14	8	45	6	41
28	8	34	7	51
	Muck			
0	48	12	0	40
7	13	7	4	76
14	12	25	2	61
28	6	7	5	82
	Quartz sand			
0	>99	0	0	< 1
8	61	0	39	< 1
16	22	0	78	< 1
32	12	0	88	< 1

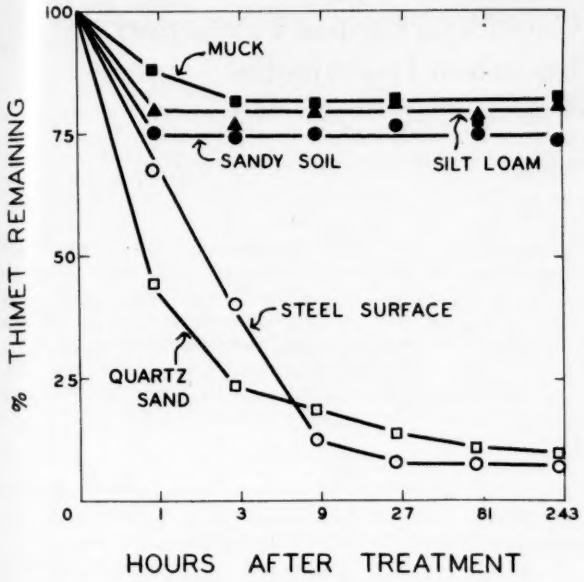

FIG. 2.—Volatilization of phorate from three soils, quartz sand, and a steel surface.

because of the position where the peak eluted, it was assumed to be a mixture of the two phorate derivatives.

Phorate applied to quartz sand behaved in a completely different manner than when applied to the agricultural soils. The phorate did not form any detectable oxidation derivatives, and owing to a lack of organic constituents most of the radioactivity was extracted from the sand. Unlike the agricultural soils, the quartz sand provided a medium where hydrolysis could proceed at a rather rapid rate.

The oxidation of phorate in soils could result from air oxidation and/or microorganism metabolism. It has been shown that certain soil organisms can oxidize and hydrolyze phorate in nutrient culture (Ahmed & Casida 1958). Attempts to sterilize soil and measure the oxidation of phorate have given inconclusive results because of the difficulty in completely sterilizing the soil without destroying its physical composition. However, there is evidence that the rate and magnitude of phorate oxidation is not affected by a large reduction in soil microorganisms. It is also possible that agricultural soils provide a catalytic mechanism for phorate oxidation since oxidative derivatives of phorate were not detected in the quartz sand.

Volatilization of Phorate from Soil Surfaces.—The volatilization of phorate from three soils, quartz sand, and a nickel-coated steel surface after a spray application is shown in figure 2. After a rapid initial loss of phorate within an hour, little or no radioactivity was lost from any of the three soils for the duration of the experiment. Following the initial loss, the sandy soil, silt loam, and muck bound approximately 75%, 80%, and 82%, respectively, of the radioactivity. Phorate was lost from the inert quartz sand and steel surface at an extremely rapid rate within the first 24 hours, after which the loss was less. The faster initial loss from the quartz sand as compared with that from the flat steel surface is probably caused by the greater surface area present in the sand. The larger surface area could also account for greater retention of radioactivity on this material later in the experiment.

DISCUSSION.—Plants can absorb greater amounts of phorate from sands and sandy soils than from heavier soils. Phorate uptake by plants from high organic matter soils is extremely poor as compared with uptake from other types. The major extractable fraction of phorate applied to soils is preserved as a mixture of the phorate sulfoxide, and sulfone, and probably converted to these forms by a chemical oxidative mechanism rather than an organism metabolism process.

Although only a very small percentage of an insecticide applied as a soil treatment is absorbed and translocated by a plant, this method of application can be useful. The soil medium acts as a protective mechanism by reducing hydrolysis and volatilization of the phorate. Longer residual effects can be obtained if correct application procedures are used. Higher rates of toxicant per acre would be required for soil treatments than for foliar applications, but at the same time one soil treatment would replace numerous foliage sprays.

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Increased Tolerance of the Boll Weevil and Cotton Fleahopper to Some Chlorinated Hydrocarbon Insecticides in Central Texas in 1958[1]

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Abstract

The boll weevil (*Anthonomus grandis* Boh.) and cotton fleahopper (*Psallus seriatus* (Reut.)) showed increased tolerance to several of the chlorinated hydrocarbon insecticides in central Texas in 1958. Topical-application studies on weevils reared from squares collected from one field in July indicated increased tolerance to toxaphene and a high tolerance to dieldrin. In field experiments much better control was obtained with Sevin® (1-naphthyl *N*-methyl carbamate) and such organic-phosphorus compounds as Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) and malathion than with toxaphene and dieldrin. Dosages of toxaphene, dieldrin, and heptachlor two and three times those effective in previous years failed to control the cotton fleahopper. Sevin, Guthion, malathion, toxaphene plus DDT, dieldrin plus DDT, and DDT alone were effective, but DDT has not been used against the cotton fleahopper for 10 years in the area.

Resistance of the boll weevil (*Anthonomus grandis* Boh.) to the chlorinated hydrocarbon insecticides in Louisiana was reported by Roussel *et al.* (1955, 1956). Resistance in a small area in Robertson County, Texas, was reported by Walker *et al.* (1956). In experiments conducted in the vicinity of Waco, Texas, against heavy late-season infestations in 1955, good control was obtained with these insecticides, although Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) was sometimes more effective (Cowan *et al.* 1956). In 1956 the chlorinated hydrocarbons continued to give satisfactory control of medium to heavy infestations (Cowan *et al.* 1957). Following a very dry fall, infestations were unusually light in 1957, and this pest was not a factor in crop production or in experiments conducted during that season (Cowan *et al.* 1958).

Difficulties in controlling the cotton fleahopper (*Psallus seriatus* (Reut.)) with several of the chlorinated hydrocarbon insecticides were reported by growers in the Lower Rio Grande Valley in 1956 and 1957. Resistance was suspected, but no research data were available to confirm it. Good control was obtained in central Texas in 1957 in experiments conducted under medium to heavy infestations (Davis *et al.* 1958).

Cotton growers have used the early-season control program in this area since its value was proven in community-wide experiments conducted in 1949 and 1950 (Ewing & Parencia 1949, 1950). The program is directed toward the control of thrips (*Frankliniella* sp.), the cotton fleahopper, and the overwintered boll weevil. Insecticides are applied at weekly intervals from the middle of May to about June 10. A high percentage of weevils has emerged from hibernation by this date, and the discontinuance of treatment allows an insecticide-free period of about a month before the bollworm (*Heliothis zea* (Boddie)) appears. This permits beneficial insects to reestablish themselves to assist in controlling this pest. If cotton is planted at the optimum time and the program is carried out properly, the crop is often produced without additional insecticide treatment. However, control measures for late-season insects are used when needed. Toxaphene is used by most growers in the program, although aldrin, heptachlor, dieldrin, and BHC, which are effective against early-season pests, have also been used.

Continuous rains during the fall months of 1957 caused plants to regrow and to produce fruit, providing ideal conditions for a late-season boll weevil buildup. Adverse weather delayed harvest of the crop and stalk destruction, permitting fruiting of plants and reproduction of weevils in large numbers until frost. The winter was mild and weevil survival was high in 1958. Weather conditions in the spring permitted only about 25% of the acreage to be planted in April.

Inspections on one farm indicated no appreciable reduction in weevil population a few days after treatment with toxaphene by the grower. Therefore, experiments were conducted in two fields to determine the reason for the failure. Soon after these experiments began, infestation records indicated that control of the cotton fleahopper was not satisfactory. Other farms were then included to determine whether difficulties in obtaining control of both pests would occur elsewhere in the area. Other pests of importance later in the season were the bollworm and the desert spider mite (*Tetranychus desertorum* Banks).

Procedure.—Previous experience had shown that small plots with replicated treatments were unsatisfactory for evaluating insecticides against these insects. Therefore, plots in all experiments were 32 to 48 rows wide and 2 to 10 acres in size.

Methods of applying insecticides early in the season were the same as those reported by Davis *et al.* (1957). Applications were made about every 7 days, although because of rain 10 days elapsed between some applications. Late-season applications were made in accordance with those reported by Cowan *et al.* (1956), at approximately 5-day intervals.

Overwintered boll weevil populations were determined by inspecting 500 to 1,000 plants per plot before and 2, 4, and 6 days after treatment. Methods of recording other data were the same as those reported by Ewing & Parencia (1943). Treatments used and data obtained in the control of early-season insects are shown in table 1 and those for late-season insects in the same experiments in table 2.

Results.—*Experiment 1.*—Records made 2 days after

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Table 1.—Effectiveness of various insecticide sprays against overwintered boll weevils and cotton fleahoppers.

Insecticide (Lb./Gal. in Concentrate)	Active Ingredient (Lb./A.)	Weevils per Acre		Weevil-Punctured Squares (%)	Fleahoppers per 100 Terminals	
		Before Treatment	After Treatment[a]		Before Treatment	After Treatment
	Experiment 1 (Applications on June 2 and 9)					
Guthion 1.5	0.25	400	4	5.4	10.1	7.3
Sevin 2.5	1.25	275	12	4.8	15.8	6.4
Toxaphene 6	2.25	325	175	19.2	15.8	21.8
	Experiment 2 (Applications on June 6 and 13)					
Toxaphene 6	2.25	150	12	24.8	25.2	31.6
Toxaphene 4+DDT 2	1.5 +0.75	175	25	16.4	22.6	9.2
Toxaphene 6+malathion 5[b]	1.5 +0.62	200	0	9.0	25.0	11.8
Malathion 5[c]	1.25	75	0	11.4	19.0	11.4
Monsanto CP-7769, 2[c]	0.5	150	12	16.5	19.0	12.5
Dieldrin 1.5[c]	.25	250	88	34.1	16.8	11.2
	Experiment 3 (Applications June 4, 10, and 20)					
Toxaphene 6	1.5	200	125	30.2	58.8	43.3
Sevin 2.5	0.62	100	62	9.2	52.6	34.6
Untreated	—	100	162	46.8	49.6	123.2
	Experiment 4 (Applications on June 4 and 11)					
Toxaphene 6	1.5	50	108	8.5	17.0	24.6
Toxaphene 6+malathion 5	1.5 +0.62	150	0	4.8	17.4	7.9
Malathion 5	1.25	175	17	3.8	20.6	12.2
	0.62	25	17	2.9	5.6	11.6
Dicapthon 2	1.0	200	0	8.2	19.8	14.7
Untreated	—	75	192	41.5	13.4	35.4
	Experiment 5 (Applications on June 10 and 20)					
Toxaphene 6	1.5	50	75	22.5	102.2	36.1
Toxaphene 6+malathion 5	1.5 0.16	100	25	10.2	77.6	13.2
	1.5 +0.31	125	50	14.6	69.2	10.7
	1.5 + .62	250	100	14.7	55.4	8.1
Untreated	—	—	88	24.0	—	27.2
	Experiment 6 (Applications on June 10 and 20)					
Toxaphene 4+DDT 2	1.5 +0.75	350	75	17.0	71.2	4.1
Toxaphene 6	2.25	50	50	22.5	72.8	29.5
	Experiment 7 (Applications June 23 and July 3)					
Toxaphene 4+DDT 2	1.0 +0.5	—	—	—	94.3	13.4
DDT 2	0.75	—	—	—	69.0	19.8
Dieldrin 1.5	.2	—	—	—	83.6	30.0
Heptachlor 2	.25	—	—	—	83.4	30.8
Untreated	—	—	—	—	67.7	40.4

[a] One day before second application in experiments 5 and 6.
[b] 0.5 pound of DDT added in second application for bollworm control.
[c] 0.75 pound of DDT added in second application for bollworm control.

the initial treatment showed a high kill of overwintered weevils with Guthion and Sevin® (1-naphthyl *N*-methyl carbamate), but a low kill with toxaphene. Four days after treatment no weevils were found in the plot treated with Guthion, very few in the one treated with Sevin, and a reduction of only 65% in the plot treated with toxaphene. By the sixth day more weevils had migrated into the plots, and they were retreated on June 9. Guthion and Sevin gave good cotton fleahopper control, but toxaphene did not.

On June 23 the boll weevil infestation averaged 8.4%, 7.6%, and 22.6% of punctured squares in the plots treated with Guthion, Sevin, and toxaphene, respectively. Because toxaphene plus DDT is more effective in bollworm control than toxaphene alone, it was used in the late-season applications (table 2) in the plot initially treated with toxaphene. Although weevil control was not needed at that time in the plots treated with Guthion and Sevin, bollworm infestations were sufficiently heavy to warrant control and treatment was made, but DDT was added to Guthion. By July 8 the weevil infestation in the plot receiving toxaphene plus DDT had increased considerably. In subsequent applications the dosage was increased to 3 pounds of toxaphene plus 1.5 pounds of DDT. For the same reason the dosage of Sevin after July 14 was increased from 1.25 to 1.5 pounds. Good control was obtained with Guthion plus DDT and Sevin, but toxaphene plus DDT was less effective. All treatments gave good bollworm control.

In the first yield record, made on August 26, about 300 and 400 more pounds of seed cotton per acre were picked from the Guthion- and Sevin-treated plots, respectively, than from the plot treated with toxaphene. This reflected the difference in control of overwintered weevils and cotton fleahoppers obtained with the materials early in the season. The plants in the plot initially treated with

Table 2.—Effectiveness of various insecticides applied as sprays against late-season boll weevil, bollworm, and spider mite infestations and yields of seed cotton.

Insecticide (Lb./Gal. in Concentrate)	Active Ingredient (Lb./A.)	Spider Mites per Sq. In.	Weevil-Punctured Squares (%)	Bollworm-Injured		Seed Cotton per Acre (Lb.)	
				Squares (%)	Bolls (%)	Total	Gain Over Check
Experiment 1 (10 applications between June 23 and August 6)							
Guthion 1.5+DDT 2	0.25+1.0	0	8.8	3.8	3.6	1,998	—
Sevin 2.5	1.4	0.22	17.5	2.9	2.9	2,041	—
Toxaphene 4+DDT 2	2.7 +1.35	.10	40.3	3.2	3.3	1,702	—
Experiment 2 (10 applications between June 26 and August 6)							
Toxaphene 4+DDT 2+malathion 5	2.0 +1.0 +0.31	0.15	36.7	4.5	3.8	1,389	—
	2.0 +1.0 +0.62	0	17.6	3.1	2.8	1,836	—
Toxaphene 4+DDT 2	2.0 +1.0	0.18	37.7	3.1	3.4	1,678	—
Malathion 5+DDT 2	1.6 +1.0	0	22.4	3.4	3.5	1,951	—
Monsanto CP-7769, 2+DDT 2	0.7 +1.0	0	30.1	3.6	2.6	1,915	—
Dieldrin 1.5+DDT 2	.5 +1.0	0	64.9	4.4	4.8	1,449	—
Experiment 3 (4 applications between June 27 and July 17)							
Toxaphene 4+DDT 2[a]	2.0 +1.0	0.12	25.3	1.9	1.7	892	583
Sevin 2.5[a]	1.25	.15	11.2	1.3	1.8	850	541
Untreated	—	.17	47.8	1.8	3.0	309	—
Experiment 4 (3 applications between June 26 and July 8)							
Toxaphene 4+DDT 2[a]	2.0 +1.0	0.8	15.0	2.0	1.2	758	389
Toxaphene 4+DDT 2+malathion 5	2.0 +1.0 +0.62	1.5	14.1	1.8	1.5	848	479
Malathion 5+DDT 2	1.25+1.0	1.8	8.9	1.1	1.4	862	493
	0.62+1.0	1.1	9.6	1.0	2.0	761	392
Dicapthon 2+DDT 2	1.0 +1.0	0.3	10.8	1.7	2.0	889	520
Untreated	—	.06	54.2	1.4	2.8	369	—
Experiment 5 (5 applications between June 27 and July 17)							
Toxaphene 4+DDT 2	2.0 +1.0	0.6	12.8	3.5	4.0	888	233
Toxaphene 4+DDT 2+malathion 5	2.0 +1.0 +0.16	.6	13.3	3.5	5.2	830	175
	2.0 +1.0 + .31	.8	10.7	3.2	3.8	915	260
	2.0 +1.0 + .62	1.1	9.2	2.9	4.3	868	213
Untreated	—	0.7	46.0	5.4	15.3	655	—
Experiment 6 (5 applications between June 27 and July 17)							
Toxaphene 4+DDT 2	2.0 +1.0	0.5	12.0	3.5	5.0	1,002	—
Toxaphene 4+DDT 2+malathion 5	2.0 +1.0 +0.31	.6	12.5	3.6	3.5	819	—
Experiment 7 (7 applications between July 8 and August 5)							
Toxaphene 4+DDT 2	2.0 +1.0	0.3	10.5	2.2	3.4	1,445	1,010
Malathion 5+DDT 2	0.62+1.0	1.0	7.7	3.5	5.1	1,194	759
Dieldrin 1.5+DDT 2	.38+1.0	0.4	8.7	3.2	5.1	1,043	608
Heptachlor 1+DDT 2	.5 +1.0	.3	18.3	3.1	5.2	1,046	611
Untreated	—	.4	50.3	5.3	10.7	435	—

[a] 0.125 pound of demeton added for spider mite control in first application.

toxaphene were unable to overcome later in the season the early loss of fruit, and in the final yield they produced about 300 pounds less seed cotton per acre than those treated with Guthion plus DDT or Sevin.

Experiment 2.—Good control of overwintered weevils was obtained with toxaphene plus malathion and with malathion alone (table 1). Fair control was obtained with Monsanto CP-7769 (hexaethyl (ethylthiomethylidine) triphosphonate), toxaphene, and toxaphene plus DDT, but that with dieldrin was poor. On the day preceding the second early treatment the bollworm infestation was considerably higher in the plots receiving phosphorus compounds and dieldrin than in those receiving toxaphene and toxaphene plus DDT. DDT was added to the toxaphene-malathion, malathion, CP-7769, and dieldrin treatments for this control. Toxaphene gave poor control of cotton fleahoppers, but the other materials gave good control. Twelve days after the last early-season application the percentage of weevil-punctured squares was considerably higher in the plots treated with toxaphene and dieldrin than in those receiving the other insecticides. However, because toxaphene gave poor fleahopper control, fewer squares were available than in other plots.

On June 26 the dosage of DDT was increased to 1 pound in the plots receiving mixtures with DDT, and after July 1 the plot initially treated with toxaphene was treated with toxaphene plus DDT plus malathion (table 2). Excellent late-season boll weevil control was obtained with toxaphene plus DDT plus 0.62 pound of malathion and with malathion plus DDT. Control with CP-7769 plus DDT was good and that obtained with toxaphene plus DDT and toxaphene plus DDT plus 0.31 pound of malathion was fair, but dieldrin plus DDT gave poor control. Each insecticide gave satisfactory bollworm control.

Infested squares collected from this field on July 22 were forwarded to the Texas Agricultural Experiment Station for topical-application studies on weevils reared from them. Results indicated increased tolerance of

weevils to toxaphene and a high degree of resistance to dieldrin. Yields were considerably lower in the plots initially receiving toxaphene and dieldrin, reflecting the poor control of cotton fleahoppers obtained with toxaphene and of boll weevils with dieldrin.

Experiment 3.—Toxaphene gave poor control of overwintered boll weevils and Sevin gave fair control (table 1). Sevin gave better fleahopper control than toxaphene, but the dosage was too low for maximum control of existing heavy infestation.

For late-season control (table 2) DDT was added to the toxaphene treatment and the dosage of Sevin was increased. Demeton at 0.125 pound was added to both treatments in the first late-season application for spider mite control. Sevin gave good boll weevil control, and that obtained with toxaphene plus DDT was satisfactory. Both treatments gave good control of bollworms.

On the first picking more than 500 pounds of seed cotton per acre were picked from the treated plots; the untreated check produced so few open bolls that pickers refused to pick that plot. Both treated plots gave good increases in total yield over the untreated check.

Experiment 4.—All treatments except toxaphene alone gave good control of overwintered weevils (table 1). They also gave much better control of fleahoppers.

Satisfactory late-season weevil control (table 2) was obtained with all the other insecticides previously used and with toxaphene when DDT was added. All treatments contained DDT and gave good bollworm control. Spider mite infestations developed following all early treatments except dicapthon. Infestation in the toxaphene plot was controlled by the addition of demeton to the first late-season application of toxaphene plus DDT, but the continued use of the same dosages of malathion in the other plots failed to give appreciable control. Good increases in yield over the untreated check were obtained from all treatments, but they were greater from dicapthon plus DDT, toxaphene plus DDT plus malathion, and 1.25 pounds of malathion plus DDT, than from 0.62 pound of malathion plus DDT, and toxaphene plus DDT.

Experiment 5.—Treatment was started too late for optimum overwintered-weevil control, as some of the plants were producing squares one-third grown permitting weevil oviposition (table 1). However, when the first punctured-square record was made on June 16, the infestation was higher in the plot treated with toxaphene alone than in those receiving toxaphene plus any dosage of malathion. Each of the toxaphene-malathion mixtures gave better fleahopper control than toxaphene alone, the two at the higher malathion dosages being more effective than the one at the lowest dosage. After 24 hours the fleahopper infestation was reduced in the untreated check through drift from toxaphene-malathion applied to the adjacent plot. This probably also happened in the plot receiving toxaphene. After 5 days the infestation was much higher in the plots receiving toxaphene alone and the untreated check than in those receiving the mixtures.

For late-season control (table 2) DDT was included in all the treatments. Each treatment gave good control of the boll weevil and bollworm and there were no appreciable differences in yield. The various dosages of malathion added to toxaphene plus DDT failed to prevent the development of desert spider mite infestations.

Experiment 6.—Overwintered weevil populations (table 1) were heavier in the plot receiving toxaphene plus DDT. A reduction of nearly 80% was obtained with it with the first application, but no reduction was indicated from toxaphene. Toxaphene plus DDT gave good fleahopper control, but toxaphene did not.

For late-season control (table 2) the dosage of toxaphene plus DDT was increased in the plot receiving the mixture and the plot initially treated with toxaphene was treated with a mixture of toxaphene, DDT, and malathion. Both mixtures gave good boll weevil and bollworm control. The addition of malathion failed to prevent an infestation of the desert spider mite. The plot treated with the toxaphene-DDT mixture throughout the season produced more seed cotton per acre than the plot originally treated with toxaphene, reflecting the superior control of the cotton fleahopper obtained with it earlier in the season.

Experiment 7.—This was conducted later in the season than the previous experiments to determine whether DDT, heptachlor, and dieldrin would be as effective in fleahopper control as toxaphene plus DDT, which gave good control in other experiments. Each insecticide gave good initial control of fleahoppers, but after 7 days infestations reached injurious levels in the plots receiving heptachlor and dieldrin. However, after 10 days they also reached injurious levels in the toxaphene-DDT and DDT plots and another application was made on July 3. The best seasonal control was obtained with toxaphene plus DDT (table 1). DDT alone was satisfactory, but dieldrin and heptachlor were not.

A bollworm infestation developed and late-season treatment was started for control of this insect on July 8. Toxaphene plus DDT gave somewhat better bollworm control than the other mixtures, and heptachlor plus DDT was the least effective against the boll weevil. Toxaphene-DDT gave the highest gain in yield and the other mixtures were better than heptachlor-DDT.

DISCUSSION.—The data indicate that tolerance to the chlorinated hydrocarbon insecticides in boll weevil populations in central Texas has increased to such a degree that satisfactory control may not be obtained with them under heavy infestations. Control with them in previous years was comparable to that obtained with Sevin, Guthion, and malathion in 1958. There also was an indication that toxaphene plus DDT may be more effective than mixtures of DDT with the other chlorinated hydrocarbons.

For the first time since chlorinated hydrocarbon insecticides have been used in the early-season control program, growers failed to control the cotton fleahopper. The timing of applications was good, as migration of this pest to cotton occurred when growers were applying their third early-season applications at increased dosages, and sufficient control should have been maintained to permit setting of fruit. The last application, scheduled for the following week, should have controlled the pest. This was not the case except on a few farms where toxaphene plus DDT or BHC plus DDT were used in the program. Results of experiments were similar to experiences of growers. Dosages two and three times those previously used to maintain control for a week to 10 days failed to give control. Nymphal populations increased during treat-

ment, indicating that lack of control was not due to adult migration. DDT alone and in mixtures with other chlorinated hydrocarbons previously used alone gave control. They probably continued to be effective because they have not been used for early-season control for 10 years in the area. Toxaphene, dieldrin, and heptachlor were considerably less effective against the fleahopper than in previous years. It is concluded that the fleahopper has developed considerable tolerance to these insecticides in this area.

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Distance from the Apiary as a Factor in Alfalfa Pollination[1]

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ABSTRACT

Experiments conducted in northern Utah indicated that the distribution of honey bees (*Apis mellifera* L.) on an alfalfa field is modified by various influences besides distance from the colonies. Because of these other factors, generalizations concerning the effect of distance from colonies on the distribution of foraging honey bees cannot be made on the basis of experiments herein reported.

A slight negative relationship between bee populations and distance from colonies in alfalfa fields was found in some of the experiments reported. In two fields where the distance was less than 600 feet, horizontal stratification of the field population was found only in the one with an average population of over two bees per square yard. The other field had less than one bee per square yard and no significant changes in population were observed up to 550 feet from the apiary.

In another location bee populations decreased beyond 3,000 feet, but other variable factors on the field made it impossible to attribute these decreases solely to increasing distance from the apiary.

Most alfalfa seed growers who use honey bees (*Apis mellifera* L.) for pollination are faced with the problem of how to distribute the colonies for greatest efficiency. Beekeepers have always placed their bees as close to major sources of nectar as topographical conditions and the attitudes of farmers would permit, but seldom have colonies been placed within the field.

With the increasing use of planned alfalfa pollination, apiculturists and other investigators, on the basis of limited observations, have recommended the shortest practical distance between the colonies and the individual flowers to be pollinated. As a result, various patterns of colony placement have been used. Colonies have been distributed singly, in groups, or in rows along roadways within seed fields.

There is little in the literature to indicate that recommended patterns were based on critical observations or experimental work, although Vansell (1951) observed that in California alfalfa plants within 300 feet of colonies produced 30% more seed than plants 100 feet farther away.

Several studies have been reported recently on the effect of distance from colonies on seed yields of red clover. Walstrom *et al.* (1951) and Walstrom (1958) in Iowa and MacVicar *et al.* (1952) in Canada found sharp drops in yield beyond 400 and 3,300 feet from the colonies, respectively. Zivov & Skvorkov (1951) in Russia and Braun *et al.* (1953) in Canada reported a more gradual decrease in seed yields as distance increased. Thus, while it appeared that a negative relationship existed between seed yield of red clover and distance from bee colonies, the critical distances vary widely under different conditions.

Recommendations based on experiments with red clover may not apply to alfalfa because of the wide difference in pollinating activities of honey bees on these

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Table 1.—Average number of bees per square yard at increasing distances from the apiary on two 5-acre plots at Fielding, Utah, 1950.

FEET FROM APIARY	PLOT 1	PLOT 2
0	3.23	3.77
24	2.68	3.18
48	2.41	3.14
72	2.91	3.32
96	2.23	3.09
120	2.32	3.36
144	2.54	3.32
168	2.27	3.00
192	2.45	2.82
216	2.50	2.86
240	2.91	2.54
264	2.54	2.41
288	2.41	2.59
312	2.36	2.91
336	1.64	2.68
360	2.09	2.86
384	1.95	3.04
408	2.00	2.68
432	2.32	2.23
456	2.09	2.72
480	2.09	2.86
Average	2.38	2.93
r=	−0.668**	−0.702**

* Significant at 5% level.
** Significant at 1% level.

two crops. Furthermore, the variable results with red clover indicate that more work on that crop is needed to measure some of the factors responsible for observed differences in yields at various distances from bee colonies.

Several experiments have been conducted in northern Utah alfalfa fields to determine the effect on bee populations of increasing distances from apiaries.

FIELDING, UTAH.—Observations on 150 acres of seed alfalfa in a cooperative study of alfalfa-insect control and pollination carried out in 1950 revealed highly significant negative correlations between bee populations and their distances from the hives (table 1). The average bee population on this field was relatively high, ranging from 2.4 to 2.9 bees per square yard. Counts were made on two plots at 24-foot intervals up to 480 feet away from a group of 232 colonies. These counts were made 5 days per week for 6 weeks. The data in table 1 represent the seasonal averages at each distance. Data from the field margins were deleted in these calculations to eliminate the influence of greater bloom on plants growing there.

Although the percentages of open flowers tripped at the bee-counting stations showed no trends, and seed yields were not measured in relation to distance from the colonies, the changes in bee population justified more detailed studies.

NEWTON, UTAH.—An experiment was conducted on a rectangular 14-acre field of alfalfa at Newton, Utah, in 1951. The primary objective was to determine whether within a distance of 600 feet alfalfa-seed yields and bee populations would be influenced by the distance from colonies of honey bees.

Methods.—One hundred colonies were placed in a double row across the middle of the field. Parallel to the line of bees, the field was divided into ten 100-foot sampling belts. Ten-foot buffer strips were left between each belt and at each end of the field (fig. 1). The field was

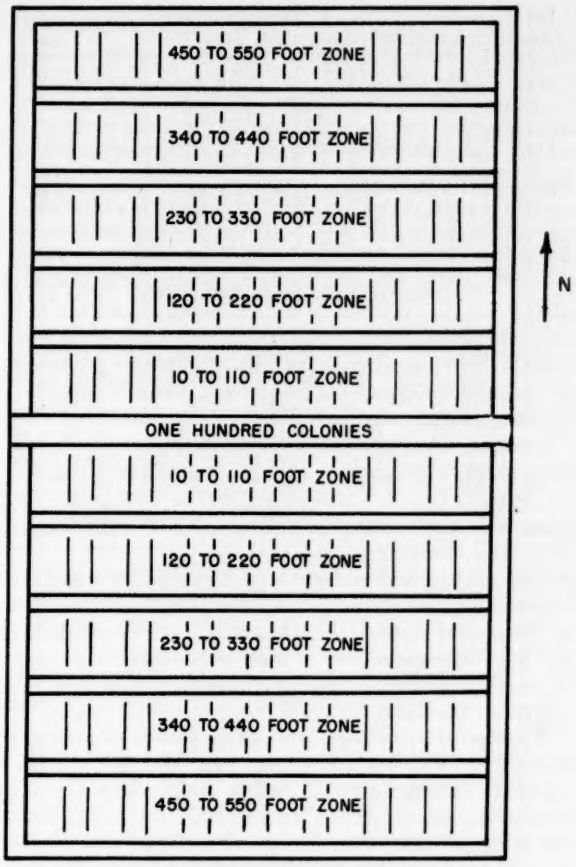
450 TO 550 FOOT ZONE
340 TO 440 FOOT ZONE
230 TO 330 FOOT ZONE
120 TO 220 FOOT ZONE
10 TO 110 FOOT ZONE
ONE HUNDRED COLONIES
10 TO 110 FOOT ZONE
120 TO 220 FOOT ZONE
230 TO 330 FOOT ZONE
340 TO 440 FOOT ZONE
450 TO 550 FOOT ZONE
N

FIG. 1.—Diagram of experimental alfalfa seed field, Newton, Utah, 1951.

further divided into eight 20-foot strips at right angles to the line of colonies, thus providing subplots for sampling purposes. This arrangement provided two replications for directions and five distance treatments with eight areas for sampling across each whole plot.

Types of Data.—The data were taken by random sampling of all the subplots during the season. The numbers of honey bees and wild bees per square yard and the per cent of flowers tripped were each determined from 13 samplings and the per cent of flowers forming pods from two samplings. Seed yields were determined at harvest. The per cent of pollen-collecting bees was determined five times and the rate of tripping by nectar collectors was measured eight times on only two randomly selected rows of subplots perpendicular to the line of colonies placed across the center of the field.

Other Conditions.—The field had been planted for hay production, and the growth was dense and rank, a condition generally considered unfavorable for seed production and bee visitation. Although there was little other alfalfa in bloom in the area during the main blossoming period of the experimental field, sweetclover along roadsides and irrigation ditches was an attractive and successful competitor. Injurious insects were kept at a low level, and weather conditions were excellent for bee activity and growth of the alfalfa.

Interference by bees other than those from colonies

Table 2.—Seed yields and bee populations at various distances from bee colonies, Newton, Utah, 1951.

Distance from Apiary (Feet)	Seed Yields (Pounds per Acre)			Number of Bees per Square Yard		
	North	South	Average	North	South	Average
10–110	75.8	83.8	79.7	0.84	0.93	0.86
120–220	72.3	84.7	78.6	0.75	0.93	0.84
230–330	76.2	83.8	80.1	0.70	0.79	0.75
340–440	87.6	94.5	91.0	0.75	0.76	0.75
450–550	111.5	100.2	105.8	0.76	0.81	0.79
Analysis of Variance of Whole Plots						
df						
Direction			1			1
Distance			4			4
Linear			(1)			(1)
Error			4			4
Mean Squares						
Direction			55.7			0.0177*
Distance			272.4*			.0071
Linear			835.9**			.0162**
Error			42.4			.0020

placed on the field did not appear to be an important factor. There were probably a few colonies of honey bees in the town of Newton, less than 2 miles south of the field, and a commercial apiary of about 60 colonies was known to be 2 miles to the southeast. Wild species of bees were scarce on the field.

Results.—The seasonal average population density for honey bees was 0.8 per square yard and for wild bees 0.013. Only 0.28% of the honey bees collected pollen, and nectar collectors were seen to trip 0.93% of the flowers visited. Seed yields for all 80 subplots averaged 88 pounds per acre, unusually low for the area.

Correlations were calculated between distance and each of the following: honey bee populations, wild bee populations, tripped flowers, rate of tripping by nectar collectors, per cent of flowers forming seed pods, and seed yields, none of which were significant. Bee populations showed a significant linear relationship to distance from the colonies, but the differences were not significant (table 2). They were higher on the southern half of the field than on the northern half, but when studied separately by means of scatter diagrams, neither half showed any more relation to distance than when considered together. Bee populations appeared to be highest on the perimeter subplots, even though most of these were 10 feet or more from the edge of the field.

Honey bee populations during the peak of bloom from July 1 to 19 were correlated on a subplot basis with seed yields ($r = +0.228^*$). At this low level of correlation, bee populations cannot be considered responsible for much of the variation in seed yields. However, none of the other factors measured showed any correlation with seed yields or with each other.

HOWELL, UTAH.—In 1955 and 1956 experiments were conducted on about 200 acres of alfalfa left for seed at Howell, about 50 miles west of Logan. The main objective was to determine what effect distances up to 1 mile from the apiary would have on the population of honey bees working alfalfa.

Experimental Area.—At this location several long, irregular, narrow alfalfa-seed fields grew in a north-south direction along one side of a road (fig. 2). One mile north

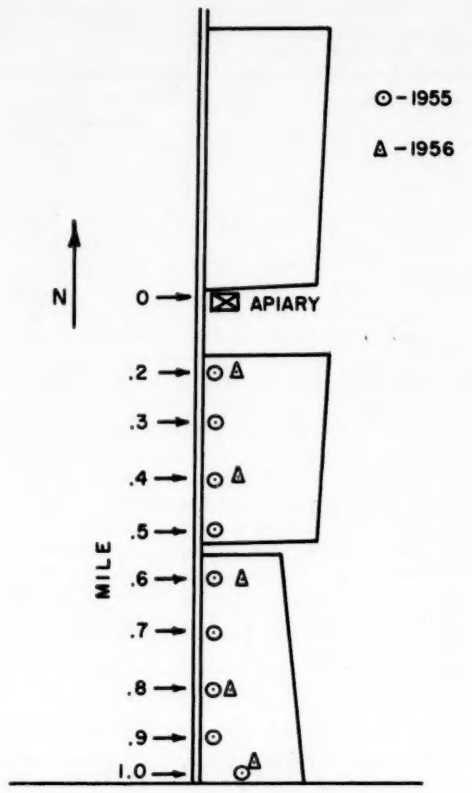

FIG. 2.—Diagram of experimental alfalfa seed fields showing sampling stations in 1955 and 1956, Howell, Utah.

of the southern edge of these fields, 160 colonies were placed in 1955 and 225 colonies in 1956. All other colonies in the area were situated more than a mile north of this apiary. Originally it was planned to take data in both directions from the bees, but the fields to the north were too far past the peak of bloom to attract enough bees to be comparable in 1955 and some of them were plowed up in 1956.

At the time measurements were taken in both seasons, these first-crop seed fields, totaling approximately 200 acres, provided most of the forage available to the bees. From 500 to 600 acres of hay alfalfa were within flight range to the north, east, and west, but only a little of this was coming into bloom in 1955 and none in 1956. Other sources of honey and pollen in the area were scarce, and only a meager crop of honey was harvested in both years.

Procedure.—In 1955 ten distances away from the bees were marked off at intervals of about 0.1 mile. The first sampling station was at zero distance, but the second station was almost 0.2 mile from the bees. In 1956 five distances were marked off at 0.2-mile intervals.

One serious drawback in using these fields was the lack of uniformity in growth and stage of bloom. This was more serious in 1955 than in 1956. However, measurements were taken in growth as nearly uniform as possible within a few feet of each measured distance.

In 1955 data collection was limited to counting the number of bees per square yard and determining the per cent of bees collecting pollen. In 1956 measurements were also made of soil surface temperature, relative humidity,

Table 3.—Honey bee populations and per cent pollen collectors at different distances from the apiary. Howell, Utah, 1955.

DISTANCE FROM APIARY (FEET)	NUMBER OF BEES PER SQUARE YARD	PER CENT OF POLLEN COLLECTORS
0	1.62	2.2
1000	1.37	3.2
1500	1.35	4.4
2100	1.56	3.4
2600	1.25	4.3
3100	1.74	2.8
3600	1.32	3.3
4100	1.26	2.5
4700	1.18	4.2
5200	1.23	5.1
Average	1.39	3.54
	Analysis of Variance	
df		
Distance	9	9
Linear	(1)	(1)
Error	80	110
Mean Squares		
Distance	0.35*	11.2*
Linear	0.86*	17.8**
Error	0.17	4.8

light reflected from a gray card on the ground, and the sugar concentration in nectar taken from the honey stomachs of bees.

In 1955 the counts were made by five observers over a period of 4 days during the peak of bloom and were made at each distance 7 to 12 times. In 1956 the counts were made by five observers over a 3-day period during peak bloom and were made at each station 19 times. The observers started at different distances on each day of counting.

In 1956, ten sets of ecological measurements were made on each day and 15 bees were examined at each distance.

Results.—In 1955 (table 3) there was a gradual decrease in bee populations as the distance increased. The per cent of bees collecting pollen increased with the distance from the colonies, especially at the greatest distance.

In 1956 (table 4) there was a decrease in bee populations beyond the 3,000-foot distance. The per cent of pollen-collecting bees showed a gradual increase as the distance from the hives increased up to 4,000 feet and then dropped slightly at the last sampling zone.

The sugar concentration of nectar taken from field bees, temperature, and light measurements all increased with distance from the hives, but the relative humidity dropped. Correlations between each of these four measurements and the per cent of pollen collectors are as follows:

	r
Sugar concentration of neetar	+0.774*
Temperature	+0.923**
Light	+0.918*
Relative humidity	−0.594*

DISCUSSION.—At first glance the different population densities at each location indicate that the influence of distance on bee populations depends on the number of bees on the field. If stratification of the bee population had appeared on fields with a small average number of bees, disappearing as the population increased, it would be easy to accept such a hypothesis. However, the fact that stratification was apparent only as the population increased suggests that other factors were involved.

The data collected at Howell afford a glimpse of the complications present in work of this type. A decrease in bee population was observed as the distance from the apiary exceeded 3,000 feet. However, the ecological data collected in 1956 show differences in the environment around the plants, indicating a change in type of stand, which could have influenced the distribution of bees as much as distance from the hives. The reasons for these ecological differences are unknown, but unmeasured changes in soil fertility or moisture could be responsible.

The increase in per cent of pollen-collecting bees which was correlated with the changes in the ecological measurements, confirms previous observations that pollen collection in alfalfa is affected by the condition and environment of the plants. As light and temperature increased and relative humidity decreased, the plants became more attractive to pollen collectors. As the plants became more attractive to pollen collectors, they may have become less attractive to nectar collectors, presumably because of insufficient water for optimum nectar secretion. This possibility tends to confound the relationship between populations and distance.

The increase in sugar concentration of the nectar removed from bees was probably a reflection of the smaller population in combination with the lower relative humidity. With fewer bees to remove the nectar, it remained in the flowers, where it was subject to concentration by evaporation.

At Newton, under rather unfavorable conditions for seed production, no stratification of the bee population was observed within the distance available, and the seed yields increased slightly with distance from the hives. Although this is contrary to the findings of investigators studying red clover, the lack of correlation between the yields and most of the pollination data indicates that at these low levels of bee populations and seed yields, other variables between plots may have masked the effects of any possible differences in bee activities.

The correlation of bee populations during the peak of bloom with seed yields at Newton indicates that measurements taken within the period of maximum activity would be more likely to reveal existing relationships than would counts extended through the entire blooming period. Consequently, in the experiments conducted after

Table 4.—Averaged data at progressive distances from the apiary. Howell, Utah, 1956.

DISTANCE FROM APIARY (FEET)	NO. OF BEES PER SQ. YD.	PER CENT OF		ENVIRONMENT AT FLOWER LEVEL		
		Pollen Collectors	Sugar in Nectar from Bees	Humidity (%)	Temperature (° F.)	Light (Ft.-C.)
1000	2.02	3.7	48.2	13.3	94.2	138.5
2000	1.74	5.8	50.6	13.6	96.0	139.2
3000	1.98	8.3	52.3	11.7	98.5	178.0
4000	1.74	11.4	53.4	9.8	100.6	193.3
5000	1.28	9.7	54.9	9.0	100.9	204.2
Average	1.75	7.78	51.9	11.5	98.0	170.6
		Analysis of Variance				
df						
Treatment	4	4	4	4	4	4
Linear	(1)	(1)	(1)	(1)	(1)	(1)
Error	90	90	220	145	145	145
Mean Square						
Treatment	1.62**	178.2**	300.8**	124.5**	254**	27,875
Linear	4.2**	588.5**	1180.9**	461.3**	972.0**	103,230**
Error	.395	29.0	19.1	13.5	43.1	1,802

1951 counts were confined to the period with the highest bee populations.

The slightly larger populations and the significantly higher seed yields on the peripheral subplots at Newton may indicate that bees tend to accumulate along the borders of fields if they go beyond and find nothing on which to work.[3] Increased attractiveness of bloom at the edge of the field was eliminated by avoiding the edge in the counts.

In view of the variable results reported here, it appears that the distribution of bees over an area is subject to modifying influences besides that of increasing distance from the colonies. Whether bees are collecting nectar or pollen, the availability of other food sources beyond the experimental field, and variations in the type of growth on the field may affect the distribution of field populations. It is important that all these factors be considered in attempts to determine the influence of distance on bee populations in the field. Because of the variations in these factors, the results reported do not provide sufficient evidence on which to base a recommendation for the most efficient distribution of colonies around alfalfa fields to be pollinated. However, it seems obvious that the closer colonies are to flowers where the bees are working, the less time and energy will be used in flying and the more pollination will be accomplished.

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[3] F. E. Todd, in correspondence, reports a simular accumulation of bees at the ends of rows of cantaloupes in Arizona.

Studies on the Metabolism and Residues of P^{32}-Labeled Delnav in a Hereford Steer[1]

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ABSTRACT

P^{32}-Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate)) was applied as a spray to a Hereford steer and the residues and metabolic pathway were determined. Fatty tissues accumulated small amounts of the insecticide, but 7 days after treatment most of the dose was still on the hair. No residues were found in meat samples. The metabolic degradation of the insecticide in mice was not affected by the route of administration. Paper and alumina chromatography demonstrated the presence of phosphate and/or phosphorothiolate compounds in some of the minor fractions of technical Delnav.

Delnav®, also known as Hercules AC-528, has recently been shown to control several ectoparasites of livestock. The technical material is a mixture of the *cis* and *trans* isomers of 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethylphosphorodithioate) and related compounds (Diveley *et al.* 1959). Analysis of residues in plant materials has been accomplished by a colorimetric method (Dunn 1958) and residues on citrus have been measured (Gunther *et al.* 1958). The metabolism of the labeled insecticide in rats and cockroaches has been studied (Arthur & Casida 1959), and the mechanisms of residue loss from plant foliage have been described (Casida & Ahmed 1959).

The studies reported herein were conducted on the metabolism and residues associated with spray application of the labeled technical compound to a Hereford steer. Data were also obtained on the metabolism of the insecticide in mice and certain of its chemical properties.

MATERIALS AND METHODS.—*Radioactive Compound.*—A 30% emulsifiable concentrate prepared from the labeled technical insecticide was supplied by the producer. An aliquot of the radioactive sample was saved for further study, and the remainder diluted with sufficient nonlabeled emulsifiable concentrate to make 1 gallon of 0.15% spray. At the time of treatment the diluted compound had an observable specific activity of 1.03×10^9 c.p.m./gram.

Experimental Animal.—The insecticide was applied to a 167-kg. Hereford steer with a power sprayer at 200 pounds' pressure in a sealed chamber. After treatment the chamber was rinsed with water, the runoff and rinses were pooled, and the applied dose was calculated by difference. Of the applied spray, 75.5% was recovered as runoff and the remainder, 1.48 grams (8.8 mg./kg.), represented the amount of insecticide actually applied.

Following treatment the animal was held in a metabolism stall, and food and water were made available. Quantitative collection of urine and feces was made throughout the experimental period, and blood and hair samples were collected at intervals. After 7 days the animal was sacrificed and tissue samples were taken for analysis.

Methods of Analysis.—Paper chromatography using acetonitrile:water (85:15) on untreated strips of Whatman No. 1 paper (Kaplanis *et al.* 1959) was used for chromatography of the hydrolytic metabolites. Chromatographs were developed to a height of 20 cm. in unlined corked glass cylinders. The methods of Hanes & Isherwood (1949) and the 2,6-dibromo-*N*-chloro-*p*-

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quinoneimine reagent described by Menn *et al.* (1957) were used to detect spots on developed chromatographs. Radioactive samples of biological origin were chromatographed with known compounds for detection of possible R_f shifts.

Column chromatography by the Celite column method of Arthur & Casida (1959) was used to fractionate technical Delnav into its components. Chromatographs were developed with *n*-heptane four-fifths saturated with acetonitrile, acetonitrile, and methanol.

Alumina chromatography as described by Dunn (1958) was used in the characterization of Delnav residues in tissues and in studying the composition of the different fractions of the technical material. The column was modified so that chloroform and methanol were passed through the column after hexane and benzene. It has been shown that chloroform elutes the phosphate analogs of a number of phosphorothioate insecticides from alumina and that methanol elutes phosphoric acid hydrolysis products (Krueger *et al.* 1959).

Erythrocyte cholinesterase activity in the blood of the treated steer was determined by the colorimetric method described by Robbins *et al.* (1958). All cholinesterase determinations were made immediately after collecting the blood samples.

Radiometric measurements and determinations of total radioactivity in blood, urine, feces, and tissue samples were made by methods described by Robbins *et al.* (1956). Organosoluble radioactivity in blood and feces samples was determined by extracting 20% suspensions with hexane and analyzing plated aliquots of the hexane extract. Control analyses showed that 98% of the radioactivity in technical Delnav was recovered in the hexane fraction when partitioned in this way. Urine samples were extracted directly with hexane. All counts were corrected for decay and self-absorption and the results expressed as microgram equivalents per gram or milliliter of Delnav.

The tissue samples were fractionated as follows: 50-gram samples were cut into small pieces and ground in a Waring Blendor in 100 ml. of acetone. The acetone was separated from the residue by filtration on a Buchner funnel and the residue reextracted with 100 ml. of benzene, which was filtered in the same manner as the acetone. For fat samples the order of solvent extraction was reversed.

The residue was suspended in water and aliquots counted to determine the unextractable radioactivity. The acetone and benzene extracts were pooled and concentrated to dryness on a hot-water bath, a stream of air being used to hasten solvent removal. The residue was partitioned with water and hexane (Skellysolve B), the water fraction representing hydrolysis products. The insecticide was recovered from hexane by two extractions with acetonitrile. Control analyses indicated better than 95% recovery of Delnav from four different tissues by this method. The acetonitrile extract was then concentrated and chromatographed on alumina as previously described.

Hair samples were obtained by pooling 25-mg. samples collected from four areas on the animal's body. They were extracted for 24 hours with 10 ml. of chloroform. The chloroform was then partitioned with water. Aliquots of both phases were analyzed to determine the total insecticide remaining on the body of the treated animal and the per cent hydrolysis. At the time the animal was sacrificed additional samples were chromatographed on alumina and Celite as described previously.

The nature of the hydrolytic metabolites formed from Delnav in white mice and possible differences owing to method of ingestion were studied by treating adult female mice with the column-purified *cis* and *trans* isomers of Delnav orally, dermally, and subcutaneously at 25 mg./kg. The mice were held in metabolism cages (Comar 1955) and 0- to 24-hour urine samples were analyzed by paper chromatography.

An attempt was made to characterize the unidentified components of technical Delnav (fractions Ia, Ib, Ic, V, and VI as described by Arthur & Casida, 1959) and the *in vitro* hydrolytic metabolites of all the fractions by characterizing their alkaline hydrolysis products. Aliquots of the various fractions eluted from a Celite column were hydrolyzed with 1 M potassium hydroxide in 50% ethanol at room temperature for 20 hours. The products were then characterized by paper chromatography as previously described.

RESULTS.—*Paper Chromatography.*—Acetonitrile: water gave excellent separation of the salts of the three major breakdown products of Delnav-diethyl phosphoric, diethyl phosphorothioic, and diethyl phosphorodithioic acids. The potassium salts of these acids had R_f's of 0.25, 0.44, and 0.63, respectively. No other hydrolytic metabolites were encountered in the alkaline hydrolysis studies, and only small amounts of radioactivity running with different R_f's were found as products of the *in vivo* metabolism in the treated steer and in mice as determined by chromatographs of urine samples.

Blood.—The results of the blood analysis from the treated steer are given in table 1. Only trace amounts of Delnav and metabolites were present at any time. Organosoluble radioactivity was below levels of counting sensitivity within 12 hours, and total radioactivity was not measurable after 36 hours. Erythrocyte cholinesterase showed a maximum depression to about 80% of pretreatment activity after 12 and 18 hours.

Excretion.—The results of the analysis of urine and feces are presented in table 2. Peak levels of radioactivity occurred at about 12 hours in the urine and at 24 hours in

Table 1.—Radioactivity in blood and RBC ChE activity of a Hereford steer following spray application of P^{32}-Delnav.

Hours After Treatment	μg.-Equiv. per Ml.		Per Cent Pretreatment RBC ChE Activity
	Total	Hexane-Soluble	
4	0.31	0.08	92.2
8	.17	.03	103.4
12	.16	<.01	81.9
18	.12	<.01	80.2
24	.07	<.01	94.8
36	.03	<.01	99.1
48	<.01	<.01	91.4
72	<.01	<.01	92.2
96	<.01	<.01	94.0
120	<.01	<.01	112.1
144	<.01	<.01	89.7
168	<.01	<.01	94.0

Table 2.—Radioactivity in urine and feces of a Hereford steer following spray application of P^{32}-Delnav.

Hours After Treatment	Urine		Feces	
	μg.-Equiv. per ml.	Cumulative Per Cent of Applied Activity	μg.-Equiv. per gram	Cumulative Per Cent of Applied Activity
6	56.1	1.32	0.1	0.01
12	61.6	2.87	1.1	.07
18	37.9	3.99	—	—
24	22.4	4.67	1.5	.17
36	15.8	5.55	—	—
48	9.7	6.41	0.4	.23
72	6.6	7.16	.3	.25
96	6.0	7.81	.3	.28
120	7.3	9.03	.7	.42
144	4.9	9.88	.6	.64
168	3.8	10.56	.5	.73

the feces. Only trace amounts of organosoluble compounds were present in the feces, none in the urine. Paper chromatography of the urine (table 3) showed that diethyl phosphorothioic acid was the major hydrolytic metabolite, indicating that cleavage of the P—S bond was the main site of hydrolytic attack. Satisfactory chromatographs on urine samples were not obtained after 48 hours, since the large amount of urine necessary to obtain adequate counts interfered with development of the chromatographs.

Tissues.—Results of the analysis of tissue samples for total radioactivity (table 4) show that, with the exception of hair, hide, and liver, no tissue had total residues higher than 0.5 p.p.m. In the various meat samples residues were below levels of detectability (0.05 p.p.m.).

Results obtained upon fractionation of a series of tissues are given in table 5. Only fatty tissues, hair, and hide had significant residues that were characterized as Delnav, based on extraction from hexane with acetonitrile, and elution from alumina columns with benzene. Residues of technical Delnav were not detectable in meat samples.

Both radiometric and chemical analyses of duplicate fat and muscle samples were performed by the Hercules Powder Company. Their results indicated lower residues than those reported here, but were generally similar, showing detectable insecticide residues in fat samples only.

Based on alumina chromatography, evidence was not obtained for the *in vivo* conversion of Delnav to a phosphate or phosphorothiolate derivative, since in no case was any large fraction of the acetonitrile-soluble radioactivity eluted from alumina with chloroform. In the tissue samples where the per cent recovery from alumina in the benzene fraction was low, much of the remaining radioactivity was eluted with methanol, indicating that trace

Table 3.—Metabolic products in urine of a Hereford steer sprayed with P^{32}-Delnav as determined by paper chromatography.

Hours After Treatment	Per Cent Metabolites as—			
	Diethyl Phosphoric Acid	Diethyl Phosphorothioic Acid	Diethyl Phosphorodithioic Acid	Unidentified
0–6	21	50	18	11
6–12	34	45	15	6
12–18	33	51	10	6
18–24	36	51	7	6
0–48 composite	34	43	14	9

Table 4.—Total radioactive compounds in tissues of a Hereford steer 7 days after spraying with P^{32}-Delnav.

Tissue	μG.-Equivalents per Gram	Tissue	μG.-Equivalents per Gram
Hair	1531.0	Brain	<0.05
Hide (shaved)	6.10	Tongue	< .05
Liver	2.60	Fat, subcutaneous	.30
Kidney	0.42	omental	.26
Lung	.17	perirenal	.24
Hard bone	.28	Marrow	.23
Soft bone	.30	Muscle, round	< .05
Bile	.22	shoulder	< .05
Heart	<.05	rump	< .05
Spleen	<.05	loin	< .05
Spinal cord	<.05		

amounts of water-soluble radioactivity had been recovered in the acetonitrile extracts.

Hair.—Analysis of hair samples showed that the major portion of the insecticide remained on the body surface. Total chloroform-extractable radioactivity in samples of hair declined from 1953 p.p.m. at 6 hours to 1363 p.p.m. at 7 days, indicating that the insecticide was absorbed at a very slow rate. In all samples less than 3% of the extractable activity partitioned into water from chloroform, indicating that little hydrolysis of the applied insecticide took place on the body surface. Comparison of a Celite chromatograph of the radioactivity extractable from the hair with chloroform with the chromatograph of a sample of the Delnav applied to the test animal showed that the percentage of the *cis* and *trans* fractions on the body surface was higher at the time of sacrifice (76%) than at the time of treatment (69%), indicating that these fractions, the most stable components of Delnav, were less rapidly volatilized or metabolized.

Studies with Mice.—The results of chromatographs of the hydrolytic metabolites obtained when mice were treated orally, dermally, and subcutaneously with corn oil solutions of purified *cis* and *trans* Delnav (table 6) indicate that the site of hydrolytic attack did not vary with the method of treatment.

Chemical Studies.—A sample of Delnav identical to that used in treating the steer was chromatographed on a Celite column. The composition of the insecticide based on radioactivity is shown in table 7. All fractions partitioned better than 20:1 into chloroform from water with

Table 5.—Fractionation of residues in certain tissues of a Hereford steer 7 days after spraying with P^{32}-Delnav.

Tissue	μG.-Equivalents per Gram				Per Cent of Acetonitrile Fraction Chromatographed as Delnav
	Residue	Water Soluble	Hexane Fraction	Acetonitrile Fraction[a]	
Hair	168.3	43.8	32.1	1286.8	94.8
Hide	4.12	1.57	0.01	0.39	83.9
Liver	1.97	0.55	.06	.03	44.2
Kidney	0.25	.15	.01	.01	37.8
Fat, subcutaneous	.06	<.01	<.01	.23	96.7
omental	<.01	<.01	<.01	.25	95.2
perirenal	<.01	<.01	<.01	.23	93.8
Marrow	<.01	.01	<.01	.22	95.8
Muscle, round	.02	<.01	<.01	<.01	79.8

[a] Equivalent to p.p.m. of technical Delnav.

Table 6.—Nature of hydrolytic metabolites in 0 to 24-hour urine of mice treated by different methods with purified *cis* and *trans* P-32-Delnav at 25 mg./kg.

Treatment	Per Cent of Hydrolytic Metabolites as—			
	Diethyl Phosphoric Acid	Diethyl Phosphorothioic Acid	Diethyl Phosphorodithioic Acid	Unidentified
Oral	11	61	8	20
Dermal	20	62	3	15
Subcutaneous	19	64	2	15

the exception of fraction VI, where it was shown by secondary partition coefficients and paper chromatography that at least two compounds were present.

When chromatographed on alumina, the *cis* and *trans* fractions (III and IV) were eluted almost entirely with benzene; the others to a lesser degree. In all fractions most of the radioactivity not eluted with benzene was eluted with chloroform, indicating that phosphate and/or phosphorothiolate compounds were present. This was further indicated by the alkaline hydrolysis studies in which diethyl phosphoric acid, which could have been formed only by hydrolysis of compounds containing the P—O moiety, was recovered as a hydrolysis product of all the fractions previously shown to possess fairly high *in vitro* anticholinesterase activity (Arthur & Casida 1959). Upon hydrolysis of the *cis*, *trans*, and *dioxene* fractions both diethyl phosphorothioic and diethyl phosphorodithioic acids were recovered, indicating that hydrolysis can occur at either the P—S or the S—C bond. No evidence was obtained for the occurrence of hydrolysis of an O—alkyl bond in either the *in vitro* or *in vivo* experiments.

DISCUSSION.—According to Arthur & Casida (1959), fatty tissues were the only tissues encountered having any tendency to accumulate Delnav. They also demonstrated by subacute feeding studies that residues in the fat were rapidly lost after Delnav feeding was discontinued.

In the studies reported here large amounts of insecticide present on the hair of the treated animal and the low rate of excretion in the urine and feces indicate that, after the first few hours at least, the rate of absorption was very low. This is in agreement with results obtained with Bayer 21/199 (Krueger *et al.* 1959, Robbins *et al.* 1959).

The metabolic degradation of the insecticide occurred primarily by cleavage of the P—S and S—C bonds to yield diethyl phosphorothioic and phosphorodithioic acids. This, coupled with oxidation of the P—S grouping, either before or after hydrolysis to yield diethyl phosphoric acid, accounted for all the *in vitro* and nearly all the *in vivo* hydrolysis products.

The results obtained by alumina chromatography of the different fractions of technical Delnav indicate clearly that P=O compounds are present in some of the minor fractions of the technical insecticide. All the fractions reported by Arthur & Casida (1959) to be active cholinesterase inhibitors were shown to be partially eluted from the alumina columns with chloroform. Paper chromatography of the alkaline hydrolysates of the same fractions yielded diethyl phosphoric acid as one of the products, which is further evidence of the presence of P=O compounds. Of particular interest are the results obtained with fractions V and VI where 50% or more of the products were diethyl phosphoric acid. This fact, together with their position of elution from the column, indicates that they may be the phosphorothiolate derivatives of the *cis* and *trans* fractions of Delnav.

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Table 7.—Composition and certain chemical properties of P-32Delnav used in experiment with Hereford steer.

Fraction	Per Cent of Total Based on Radioactivity	Per Cent Partitioning into Chloroform from Water	Per Cent Eluted from Alumina Column with Benzene	Per Cent of Hydrolysis Products as—		
				Diethyl Phosphoric Acid	Diethyl Phosphorothioic Acid	Diethyl Phosphorodithioic Acid
Technical	100	98.4	93	—	—	—
Ia	3.5	98.0	71	13	29	58
Ib	10.1	98.9	73	12	30	58
Ic	1.8	95.7	80	7	38	55
II (dioxene)	5.0	98.5	84	0	83	17
III (trans)	58.1	99.0	98	0	63	37[a]
IV (cis)	11.3	98.8	98			
V	8.2	97.7	8	51	34	15
VI	2.0	56.4	29	73	24	3

[a] Fractions III and IV incompletely separated by column chromatography, pooled for this experiment.

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Nicotine Residues in Field-Treated Cauliflower, Celery, Green Beans, Kale, Mustard Greens and Spinach[1]

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ABSTRACT

In these tests, normal dosages of Black Leaf 40 left no appreciable residues on green beans, celery or cauliflower curds. A slight residue occurred in cauliflower greens but it disappeared within a day or two after treatment. Kale and spinach retained moderate nicotine residues for about a week, and Texas mustard greens retained sizeable residues for nearly 2 weeks.

Nicotine has been used as an agricultural pesticide for many years. Its extremely poisonous effect on man is well known. Because of its highly volatile nature nicotine has been thought to disappear from treated plants within a short time (usually in minutes), thus leaving no hazardous residues on marketed produce. In general, experience has supported this; however, in 1954, 40 persons became violently ill from eating Texas mustard greens grown in the Los Angeles area. Analysis of these greens by the California Department of Agriculture, Bureau of Chemistry, showed large nicotine residues.

As a result of this nearly disastrous incident it seemed advisable to re-evaluate total nicotine residues on vegetable crops. Detailed experiments to establish nicotine residue on and in treated Texas mustard greens have already been reported (Gunther *et al.* 1959). Conclusions were that, contrary to generally held opinions, volatilization of nicotine can be quite a slow process with persistence half-lives averaging 4.5 days with initial deposits ranging from 10 to 50 p.p.m. on Texas mustard greens.

The present report summarizes the above-mentioned results and, in addition, provides residue data from similar tests with nicotine on five other vegetable crops: cauliflower, celery, green beans, kale, and spinach.

All field treatments were made with conventional power sprayers and dusters adjusted to provide thorough coverage of each crop. Field plots were from 14 to 20 feet wide and from 75 to 300 feet long. All treatments were replicated five times, and special care was taken to see that neighboring control plots were not contaminated. Applications were made on calm, sunny days when temperatures varied from 70° to 75° F. and the relative humidities were between 55% and 70%. Mustard greens were treated on December 12, 1955, when the plants were 6 to 10 inches tall, and plants had more than doubled in size by the last sampling period; analytical samples were 2 pounds per replicate and were not washed. Cauliflower was treated March 8, 1956, when an occasional plant was reaching about half maturity of the curd; one unwashed head was sampled per replicate, and the curd and leafy greens were analyzed separately. The spinach was treated October 1, 1956, about 2 weeks before normal harvest time; samples were 2 pounds each and were washed before being processed for analysis. Celery was treated July 23, 1956, about 3 weeks from normal harvest time; samples consisted of three heads per replicate and the bases of the celery heads were washed before processing. Green beans were treated October 23, 1956, nearly at harvest time; samples consisted of 4 pounds of nearly mature green beans per plot and were unwashed. Kale was treated June 18, 1956, when the plants were 6 to 10 inches tall, and by the time of the last sample plants had more than doubled in size; samples were 4 pounds each and were washed. Analytical methods for nicotine on and in these crops were the same as those described for the Texas mustard greens (Gunther *et al.* 1959).

Tables 1, 2, and 3 show the results which may be summarized as follows: In these tests there appears to be little, if any, hazardous residue problem with normal dosages of Black Leaf 40 with or without the addition of soap on green beans, celery, and cauliflower curds. There is a slight residue on cauliflower greens but this appears to be in a safe range within a day or two.

Kale and spinach retain moderate nicotine residues for about a week, and Texas mustard greens may retain sizeable residues for nearly 2 weeks.

Detailed studies on the Texas mustard greens and the wash-water studies on the kale indicate that nicotine residues in samples collected 1 day or later after treatment are primarily located within the plant tissue and waxes.

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Table 1.—Nicotine residue on and in field-treated Texas mustard greens grown at Riverside, California, during December 1955.

Black Leaf 40 Spray Treatment per 50 Gallons of Water	Gal. or Lb. per Acre	Residue in P.P.M.[a] Number of Days After Treatment						
		1/24	1	2	4	8	15	22
1 pint	50	25	23	24	15	6	2.0	0.7
1 pint+2 lb. Ivory soap	42	14	16	13	10	6	0.9	0.4
1 pint+0.8 lb. Dreft soap	42	19	16	12	9	4	0.3	0.1
2 pints+3 lb. Ivory soap	51	44	42	44	33	13	6.0	2.0
Nicotine dust 3%	30	14	8	6	4	2	0.8	0.5

[a] All values corrected for the background nicotine residue found in the untreated samples. They were 0.4, 0.9, 1.6, 0.8, 0.4, 0.2, and 2.1 p.p.m. for 1/24, 1-, 2-, 4-, 8-, 15-, and 22-day samples.

Table 2.—Nicotine residue on and in field-treated cauliflower greens at Riverside, California, during March 1956 (no residue found on cauliflower curds).

Black Leaf 40 Spray Treatment per 50 Gallons of Water	Gal. or Lb. per Acre	Residue in P.P.M.[a] Number of Days After Treatment				
		1/24	1	4	7	14
1 pint	125	5	2	4	0	0.5
1 pint+1 lb. Ivory soap	93	4	0	0	0	0.3
1 pint+0.4 lb. Dreft soap	107	7	2	2	0	1.0
2 pints+2 lb. Ivory soap	103	10	6	5	3	1.0
Nicotine dust 3%	105	10	7	6	0.5	4.0

[a] All values corrected for the background nicotine residues found in the untreated samples. These were 3.1, 5.0, 3.3, 5.5, and 2.6 p.p.m. for 1/24, 1-, 4-, 7-, and 14-day samples.

Table 3.—Nicotine residues on and in field-treated spinach, celery, green beans and kale in southern California during June to October 1956.

Crop and Black Leaf 40 Spray Treatment per 50 Gallons of Water	Gals. per Acre	Residue in P.P.M.[a] Number of Days After Treatment					
		1	2	3	7	14	22
Spinach							
2 pints+0.5 lb. Ivory soap	51	17	—	—	10	3	—
2 pints+activated soap[b]	59	16	—	—	8	2	—
Celery							
1 pint+1 lb. Ivory soap	48	—	1	—	1	0.6	—
1 pint+activated soap[b]	40	—	2	—	1	0.5	—
Kale[c]							
2 pints+2 lb. Ivory soap	60	—	10	—	—	2	0.7
2 pints	66	—	8	—	—	2	0.5
Green Beans							
2 pints+0.5 lb. Ivory soap	44	0.4	—	0.6	—	—	—

[a] All values corrected for the background nicotine residues found in untreated samples. These were 1.1, 0.4, and 0.4 p.p.m. for 1-, 7-, and 14-day samples of spinach; 0.5, 0.3, and 0.2 p.p.m. for 2-, 7-, and 14-day samples of celery; 0.6, 0.8, and 0.2 p.p.m. for 2-, 14-, and 21-day samples of kale; 0.5 and 0.4 p.p.m. for 1- and 3-day samples of green beans.

[b] The Black Leaf 40 used in these tests was commercially prepared and contained 8% Atlox 3300.

[c] In separate tests, wash water from kale samples showed 1 to 2 p.p.m. nicotine in samples collected 4 days after treatment.

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Control of Root Maggots in Swede Turnips in Newfoundland with Heptachlor and Aldrin and the Effect on Parasites and Overwintering Pupae[1]

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Abstract

Furrow applications of granulated heptachlor at 2, 3 and 4 pounds of toxicant per acre gave an average control of 78%, 88% and 89%, respectively, of root maggots in swede turnips in a 3-year period; granulated aldrin at 2, 3, 4 and 5 pounds 63%, 71%, 76% and 79%. Ninety-one to 100% of the roots in the heptachlor-treated plots and 80% to 98% of those treated with aldrin were marketable; in untreated checks, 29% to 40%.

Numbers of overwintering puparia provided a reliable index to the effectiveness of the chemicals. A highly significant correlation was noted between the number of puparia recovered and the infestation index. Both chemicals, particularly at the higher rates of application evidently caused high mortality in overwintering pupae. The insecticides eliminated the staphylinid parasite, *Aleochara bilineata* Gyll., and reduced parasitism of the pupae by the cynipid, *Trybliographa rapae* (Westw.), from 9% to less than 2%.

Root maggots are the limiting factor in commercial production of swede turnips (rutabagas) in many areas of Newfoundland. The most important species involved is the cabbage maggot, *Hylemya brassicae* (Bouché). The *H. ciliicrura* (Rond.)—*H. liturata* (Meig.) complex occurs in very limited numbers. *H. fugax* (Meig.) was collected from the roots of swedes on several occasions. Only one complete generation and a partial second develop each year in Cruciferae in Newfoundland.

This is a report on a 3-year study on furrow applications of granulated aldrin and heptachlor, at various concentrations, for control of root maggot in swede turnips. In 1956 and 1957 the effects of the insecticides on the overwintering pupae and the beneficial parasites *Trybliographa rapae* (Westw.) and *Aleochara bilineata* Gyll. were also studied.

Stitt (1953) found that aldrin and heptachlor dusts applied in the furrow with the seed at 0.03 to 0.1 pounds of toxicant per 1,000 lineal feet of row gave the most satisfactory control of root maggots in turnips in Washington. King & Forbes (1954) found that aldrin and heptachlor were more effective than dieldrin, chlordane, BHC, DDT, lindane, or parathion against root maggots in rutabagas in British Columbia.

The experiment was conducted on soils varying from stony, gravelly loam to sandy loam. They were typical of soils in which swede turnips are grown in Newfoundland. Soils in the study area contained approximately

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25% to 35% gravel, 35% to 60% sand, 13% to 25% clay and 28% to 40% silt. The organic matter varied from 2% to 9%.

MATERIALS AND METHODS.—Field plots were laid out in a randomized-block design with four replications of each treatment. A level area was selected and each plot consisted of 6 rows, each 25 feet long. Rows were spaced 28 inches apart and the plants were thinned to 9 inches apart in the row. The Wilhelmsburger variety of swede turnip was seeded directly into the treated furrow with a Planet Junior drill seeder. Seeding dates were as follows: 1955, June 21; 1956, June 14; 1957, May 30.

The rate of application for each treatment is given in table 1, the rates being based on 18,900 lineal feet of row per acre. The insecticides were applied in a 4-inch band along a furrow 1 inch deep made with an ordinary wooden marker. Applications were made by placing the amount required for a row in a graduated mason jar with a perforated lid and shaking it along the furrow.

Appraisal of Injury.—Fifty swede turnips per plot (200 per treatment) were examined at harvest in early October for maggot control. The injury to each root was assessed on the basis of the following ratings: Clean, 0; slight injury, 1; moderate injury, 2; severe injury, 4. Slight injury was indicated by some feeding on the surface, insufficient to affect the commercial value. Moderate injury consisted of maggot feeding that had penetrated the skin over a small area, the scars being removable by a limited amount of trimming. Severe injury consisted of maggot feeding that had penetrated deeply into the pulp; the severe scars could not be removed by a practical amount of trimming.

The injury rating (Stitt 1953), or infestation index (King & Forbes 1954), for each plot for each year was calculated by multiplying the number of roots in each category by its numerical value and adding the products. The maximum infestation rating possible was 200.

The percentage control (table 1) was determined as follows: the infestation index for each treatment was subtracted from that for the untreated plots and the difference was expressed as a percentage of the latter. This percentage represents the reduction in damage attributable to the treatment.

King & Forbes (1954) noted a difference of opinion among workers regarding methods of appraising injury by root maggots at harvest. Therefore, the number of overwintering puparia was tested as a basis of evaluation. This method was used experimentally in 1956 and 1957 and correlated with the infestation index method.

The numbers of puparia were estimated by carefully sifting the soil from around the roots of 50 turnips from each plot at harvest. All soil within 3 inches of each root was examined to ensure that all puparia were taken.

Effects of Chemicals on Survival of Parasites and Overwintering Pupae.—In 1956 and 1957, the puparia collected from each of the plots were held in an insectary from October to January. They were then brought into the laboratory and reared separately in vials in a desiccator. They were attended daily and all flies and parasites were removed and identified as they emerged.

Statistical Analyses.—The results were tested for significance by an analysis of variance.

RESULTS AND DISCUSSION.—The average percentage control from 1955 to 1957 (table 1) for heptachlor varied from 78 at 2 pounds per acre to 89 at 4; for aldrin from 63 at 2 pounds per acre to 80 at 5. All the treatments gave significant control (table 1). Applications of 3 or 4 pounds of heptachlor per acre each gave significantly better control than equivalent concentrations of aldrin. Heptachlor at 4 pounds was significantly better than at 2, but not better than at 3.

All the chemical treatments increased yields significantly in 1955 but not in 1956 or 1957. Heptachlor at 2, 3 or 4 pounds gave an average increase of 345, 372 and 392 marketable bushels per acre, respectively; aldrin at 2, 3, 4 or 5 pounds 249, 309, 287 and 415 marketable bushels, respectively. The percentage of marketable turnips during the 3 years varied from 91 to 100 for the heptachlor treatments and from 80 to 98 for aldrin. The untreated plots produced from 29% to 40% marketable roots; however, the majority of these were slightly injured. At $1.40 per bushel during the 3 years, the increased values of marketable swede turnips resulting from the chemical treatments varied from $423 to $640 per acre for heptachlor and from $314 to $581 for aldrin. Because of the limited amount of available land in Newfoundland, and the importance of swede turnips as a cash crop, the significance of these results may hardly be overestimated.

The numbers of overwintering puparia (table 2) provided a reliable index to the effectiveness of the chemicals. The correlation coefficients for the number of puparia recovered (table 2) and the infestation index (table 1) were: 0.63 for 1956, and 0.86 for 1957, both being significant at the 1% level.

Pupa mortality (table 3) was not significantly higher in

Table 1.—Effectiveness of furrow applications of heptachlor and aldrin against root maggots in swede turnips in Newfoundland, 1955–57.

CHEMICAL	TOXICANT PER ACRE (LB.)	INFESTATION INDEX 1955	1956	1957	Average	AVERAGE CONTROL (%)
Heptachlor[a]	2	15	28	29	24	78
	3	4	18	16	13	88
	4	6	11	19	12	89
Aldrin[b]	2	20	52	—	36	63
	3	21	40	29	30	71
	4	16	31	28	25	76
	5	—	—	25	25	80
Untreated		98	98	121	106	—
L.S.D. at the 5% level		9	13	9	7	
at the 1% level (Without check)		13	18	—	12	

[a] Granulated, 2½%; Velsicol Chemical Corporation, Chicago, Illinois.
[b] Granulated, 5%; Chipman Chemicals Ltd., Hamilton, Ontario.

Table 2.—Numbers of overwintering puparia of root maggots in rutabaga plots treated with heptachlor or aldrin at various concentrations.

CHEMICAL	TOXICANT PER ACRE (LB.)	NO. OF PUPARIA[a] 1956	1957	AVERAGE
Heptachlor	2	21	29	25
	3	11	10	11
	4	4	11	8
Aldrin	2	68	—	68
	3	70	43	56
	4	39	23	32
	5	—	35	35
Untreated		147	127	137
L.S.D. at the 5% level		36	17	20
at the 1% level (without check)		50	24	27

[a] Mean for 50 roots per treatment.

Table 3.—Percentage of dead and parasitized puparia of root maggots in rutabaga plots treated with heptachlor and aldrin at various concentrations.

Chemical	Toxicant per Acre (Lb.)	1956			1957		
		No. of Pupae[a]	Dead (%)	Parasitized (%)	No. of Pupae[a]	Dead (%)	Parasitized (%)
Heptachlor	2	80	21	1[b]	106	29	1[b]
	3	43	49	—	36	39	—
	4	17	59	—	51	27	2[b]
Aldrin	2	264	29	1[b]	—	—	—
	3	265	25	1[b]	167	26	—
	4	150	37	—	89	20	—
	5	—	—	—	120	33	—
Untreated		532	30	17[c]	840	18	12[d]

[a] Total collected from 200 roots.
[b] *T. rapae*
[c] 9% *T. rapae*; 8% *A. bilineata*
[d] 1% *T. rapae*; 11% *A. bilineata*

the chemically-treated plots than in the untreated ones, the chemicals not only controlled the maggots but also destroyed a fairly high percentage of those that reached the pupal stage for overwintering.

All the treatments had a detrimental effect upon parasitism. *Trybliographa rapae* and *Aleochara bilineata* normally parasitize a fairly high percentage of the larvae and pupae in Newfoundland; Wishart (1957) recorded parasitism of pupae as high as 61.4% in collections in 1951. Parasitism for all treatments in 1956 was less than 1%, in comparison with 17% in the untreated plots. In 1957 less than 2% of the pupae were parasitized while 12% were parasitized in the untreated areas.

Only the cynipid *T. rapae* survived the chemical treatments whereas both species were present in the untreated plots. In 1956, 52% of the 88 parasites reared from the untreated areas were of *A. bilineata*; in 1957, 86%.

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Effects of Dylox and Other Insecticides on Entomophagous Insects Attacking Field Crop Pests in California[1,2,3]

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ABSTRACT

Field tests were conducted to determine the relative toxicity of Dylox® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) and other insecticides to certain entomophagous insects which aid in regulating the population density of field crop pests in California. Dylox was applied on alfalfa hay at dosage rates of 3.6 to 11.4 ounces per acre. At higher dosages, Dylox was quite toxic to larvae of the convergent lady beetle (*Hippodamia convergens* G.-M.), *H. parenthesis* (Say), and *H. quinquesignata punctulata* LeConte, but only moderately toxic to the adults. *Nabis ferus* (Linn.), *Orius* sp., and one of the Aphidiine parasites, *Praon palitans* Muesebeck, of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), also were quite susceptible to higher dosages. At dosage rates of approximately one half pound or less, Dylox was only slightly to moderately toxic to coccinellid adults and larvae (*Hippodamia* spp. mentioned above), *Chrysopa* spp., and *Geocoris* spp. Parathion, which was used as a standard comparison material, was highly toxic to the entomophagous species at dosage rates of 3.6 to 8 ounces per acre. A DDT-toxaphene mixture (1.3 and 2.6 pounds, respectively), used to control many field crop pests in California, proved to be extremely toxic to *H. convergens*, *Geocoris* spp., *Orius* sp., *Chrysopa* spp., *Nabis ferus*, and *Sinea diadema* (Fabr.), and to syrphids. DDT applied alone (1.3 pounds per acre) was nearly as toxic to the entomophagous species as the DDT-toxaphene combination. On the other hand, toxaphene (2.7 pounds per acre) was not nearly so toxic as DDT and was far less toxic than the DDT-toxaphene mixture. Sevin® (*N*-methyl-1-naphthyl carbamate), applied at 19 ounces per acre, caused marked reductions in *H. convergens*, adults and nymphs of *N. ferus*, and adults of *Collops vittatus* (Say). Heptachlor applied at 2.4 ounces per acre was moderately toxic to adults of *Geocoris* spp. and *C. vittatus*, whereas at this dosage heptachlor had essentially no effect on *H. convergens*, and adults and immatures of *Orius* sp., *Chrysopa* spp., and *N. ferus*.

In recent years entomologists and others interested in pest control everywhere have become greatly concerned with the problems of arthropod resistance to insecticides, the frequent resurgence of pests following treatment, and the outbreaks of secondary pests following treatment. It has become evident that many of these difficulties arise from the adverse effects that widely toxic materials have on other organisms in the ecosystem to which the pest species belong. We have come to realize that the application of an insecticide does not cause a simple permanent change in the arthropod complex in an environment, but acts as a temporary selective pressure upon living organisms with intricate intra- and interdependent relationships. Such delicate relationships can be and often are drastically altered by pesticides, particularly those materials with wide toxicity spectra. It is recognized that the use of the new organic insecticides and acaricides is essential to modern agriculture and public health. Thus, the problem confronting entomologists is how best to utilize the efficient products of pesticide chemistry while preserving the biological controls that produce a lower general equilibrium position of the pest species

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[3] Recognition is expressed to Drs. Ray F. Smith and Kenneth S. Hagen who cooperated in Test No. 5 at Dos Palos, California.

than would prevail without these biological controls. (A detailed discussion of differences between chemical and biological control and other problems associated with the suppression of arthropods is found in Stern *et al.* 1959).

A major step toward solving the problem is the development of chemical control programs that complement existing biological control. Consequently, in addition to data on residues and the performance of new compounds on phytophagous species, which, in the case of Dylox® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) was presented by Reynolds *et al.* (1960), a better understanding is needed of the effects of the chemicals on entomophagous species.

To this end, the authors, while evaluating the effectiveness of Dylox on many phytophagous insects, also studied the relative toxicity of this and other materials on certain entomophagous species that attack field crop pests in California. In these tests, all the materials were applied as sprays on alfalfa hay, a highly favorable crop for such experimentation because of its normally uniform growth and the many easily sampled entomophagous insects which usually seek harbor in it.

Both airplane and ground equipment were used for application of material. Where ground equipment was used, the sprays were applied by No. 4X spraying system hollow-cone nozzles spaced 18 inches apart on a 30-foot boom for broadcast spraying. Each treatment was replicated four times. When an airplane was used, each treatment was replicated three times. The effects of the treatments on entomophagous species were evaluated by means of a standard insect-sweeping net, each sweep describing a 180° arc in the alfalfa. The first samples were taken 24 hours after treatment. Additional samples were taken within a few days after treatment to avoid the possibility of adults migrating into the treated areas.

TEST NO. 1.—On October 24, 1957, Dylox and parathion sprays were applied by ground equipment to alfalfa about 12 inches tall at Hinkley (Mojave Desert), California. The effect of the materials on beneficial insects was evaluated 1 and 4 days after application. The data obtained are summarized in table 1. Parathion was applied at 1.6 ounces per acre—an amount far below the normal commercial dosage. However, even at this low dosage, the spotted alfalfa aphid parasite, *Praon palitans* Muesebeck, and adult and immature convergent lady beetles (*Hippodamia convergens* G.-M.), *H. parenthesis* (Say), *H. quinquesignata punctulata* LeConte, and *Chrysopa* spp., *Nabis ferus* (Linn.), *Orius* sp. and *Geocoris* spp. were markedly reduced 1 day after treatment, compared with populations in untreated plots.

Dylox applied at 11.4 ounces per acre resulted in a reduction of approximately 66% in coccinellid adults and of nearly 90% in larvae 1 day after treatment. *N. ferus*, *Orius* sp., and *P. palitans* were considerably reduced. At this same dosage, Dylox caused a reduction of approximately 50% in *Chrysopa* spp. adults and *Geocoris* spp. adults and nymphs.

At 5.7 ounces per acre, Dylox was not nearly so toxic to the coccinellid adults mentioned and to nymphs of *N. ferus* as at the higher dosage. However, it was equally severe on the lady beetle larvae, *Orius* sp. adults and nymphs, and on the adults of *N. ferus*, as well as on the spotted alfalfa aphid parasite, *P. palitans*. On the other hand this lower dosage of Dylox had little effect on adult and immature forms of *Chrysopa* spp. and *Geocoris* spp.

When the plots were sampled 4 days after treatment, the temperature was low and the sky completely overcast. These adverse weather conditions caused a reduction in insect activity, and as a result in general, fewer insects were collected in all plots than were collected the first day after treatment. However, the relative differences in status of beneficial insects in all treatments had not changed notably from the first day following treatment, except for an increase in the relative abundance of *P. palitans* at both dosages of Dylox.

TEST NO. 2.—On November 4, 1957, sprays were applied by ground equipment on alfalfa at Thermal, California. The materials tested were Dylox, DDT, toxaphene, parathion, and a DDT-toxaphene combination. The alfalfa was about 18 inches tall, and approximately 10 gallons of spray liquid were applied per acre. The effect of the spray materials on the beneficial insects was evaluated 1, 2, 4, and 9 days after application. The data are summarized in table 2.

The DDT-toxaphene mixture (1.3 pounds DDT and 2.6 pounds toxaphene) was extremely toxic to a wide variety of the beneficial insect species sampled. *H. convergens*, one of the Aphidiine parasites of the spotted alfalfa aphid, *Trioxys utilis* Muesebeck, *Geocoris* spp., *Orius* sp. *Chrysopa* spp., *N. ferus*, and *Sinea* diadema (Fabr.)

Table 1.—Relative toxicity of parathion and Dylox sprays[a] to beneficial insects in alfalfa at Hinkley, California.

Treatment			No. of Beneficial Insects per 200 Sweeps at Two Intervals (Days) After Treatment[b]											
			Coccinellids		*Nabis ferus*		*Praon palitans*		*Orius* sp.		*Chrysopa* spp.		*Geocoris* spp.	
	Amount/Acre		1	4	1	4	1	4	1	4	1	4	1	4
Material	Ounces	Gal.	A–L	A–L	A–N	A–N	A	A	A–N	A–N	A–L	A–L	A–N	A–N
Parathion	1.6[c]	6.4	41–21	21–1	70–179	19–35	50	67	6–1	7–9	0–1	5–1	19–1	2–0
Dylox	11.4	8.6	66–10	50–0	39–58	17–6	73	115	2–7	7–10	34–1	25–8	18–2	6–0
Dylox	5.7	8.6	120–10	42–2	47–145	24–38	69	122	6–5	13–14	53–8	41–12	29–9	6–2
Untreated	—	—	182–92	94–26	249–430	184–182	263	143	80–20	30–16	60–10	35–10	39–3	5–0

[a] Applied by ground equipment, October 24, 1957.
[b] A=adults; L=larvae; N=nymphs.
[c] At commercial dosages (4 ounces/acre and above), parathion eliminates nearly all entomophagous insects in the treated area Stern & van den Bosch (1959) and Stern *et al.* (1958).

Table 2.—Relative toxicity of various insecticide sprays applied by ground equipment, November 4, 1957 to entomophagous insects in alfalfa at Thermal, California.

Material and Dosage per Acre	No. of Beneficial Insects per 200 Sweeps at Various Intervals (Days) After Treatment[a]															
	Hippodamia convergens				*Trioxys utilis*				*Geocoris* spp.				*Orius* sp.			
	1	2	4	9	1	2	4	9	1	2	4	9	1	2	4	9
	A–L	A–L	A–L	A–L	A	A	A	A	A–N	A–N	A–N	A–N	A–N	A–N	A–N	A–N
DDT, 1.3 lb.	5–4	11–14	10–9	12–3	3	112	110	126	3–4	68–28	175–59	70–146	19–4	52–5	26–9	25–13
Toxaphene, 2.7 lb.	32–39	56–44	45–63	68–59	88	156	161	132	13–6	56–18	88–34	26–12	29–7	35–1	23–3	9–6
DDT, 1.3 lb.+toxaphene, 2.6 lb.	6–1	8–1	5–2	8–0	5	81	86	54	3–0	32–14	92–27	22–3	8–2	32–5	15–5	9–9
Parathion, 3.6 oz.	24–4	20–2	31–2	45–3	0	55	84	108	16–7	91–32	163–48	163–119	8–1	1–1	12–2	15–3
Dylox, 7.0 oz.	44–49	43–43	81–137	108–83	51	217	256	289	4–0	54–39	124–75	161–152	16–2	21–2	18–5	22–2
Dylox, 3.6 oz.	127–89	72–65	126–161	341–151	122	259	544	397	4–7	70–27	90–36	192–99	14–1	33–2	31–4	50–5
Untreated	126–119	94–92	145–173	212–161	152	276	402	265	10–4	100–33	119–35	147–66	32–3	58–2	55–3	35–4
	Chrysopa spp.				*Sinea diadema*				*Nabis ferus*				*Syrphids*			
DDT, 1.3 lb.	5–9	2–4	5–7	3–29	4	1	8	7	1–2	5–2	12–3	10–24	1–29	1–26	0–39	0–13
Toxaphene, 2.7 lb.	18–6	13–7	9–15	25–20	22	9	9	2	21–12	22–21	27–40	27–50	11–38	3–25	0–45	0–17
DDT, 1.3 lb.+toxaphene, 2.6 lb.	2–4	4–6	1–13	5–16	0	0	2	0	1–1	2–2	2–2	4–5	0–56	0–28	0–43	0–12
Parathion, 3.6 oz.	0–4	2–1	6–1	25–4	0	0	0	3	5–9	3–5	3–2	8–7	1–5	0–8	0–4	0–0
Dylox, 7.0 oz.	13–16	21–3	12–17	27–25	22	18	20	22	5–15	15–10	26–35	25–55	0–10	1–2	0–1	0–0
Dylox, 3.6 oz.	35–16	35–13	41–15	61–12	30	15	23	28	27–49	27–43	32–62	43–98	5–13	2–2	0–6	0–3
Untreated	35–14	25–23	34–14	44–21	22	25	24	28	39–51	57–34	53–64	45–69	8–60	0–39	0–40	0–40

[a] A = adults, L = larvae, N = nymphs.

were essentially eliminated by the DDT-toxaphene spray. Adult syrphids were also eliminated, but the spray appeared to have little effect on the larvae. The results obtained in this test with DDT-toxaphene were generally comparable with those reported by van den Bosch *et al.* (1956) who tested the relative toxicity of various insecticides to predators in cotton and alfalfa fields in California.

DDT applied at 1.3 pounds per acre was nearly as toxic to the beneficial insects as the DDT-toxaphene combination. *H. convergens*, *Chrysopa* spp., *N. ferus*, *S. diadema* and *T. utilis* were largely eliminated. On the other hand, toxaphene applied at 2.7 pounds per acre was not nearly so toxic as DDT and was far less toxic than the DDT-toxaphene mixture. Parathion applied at 3.6 ounces per acre was comparable to the DDT-toxaphene mixture in its generally drastic effects on the beneficial species. Parathion killed a very high percentage of adult and immature forms of *H. convergens*, *Orius* sp., *Chrysopa* spp., *N. ferus*, and *S. diadema*, and of syrphids. Only *Geocoris* spp. appeared to survive the parathion treatment in large numbers. This fact substantiates earlier findings of Stern & van den Bosch (1959) and Stern *et al.* (1958).

Dylox applied at 7 ounces per acre was not, in general, so toxic as DDT, toxaphene, parathion, or the DDT-toxaphene mixture. However, syrphid adults and larvae were largely eliminated by both dosages of Dylox (7 and 3.6 ounces per acre). In the plots treated with the higher dosage of Dylox (7 ounces per acre) the numbers of beneficial insects had increased markedly when the plots were evaluated 4 days after application. It seems probable that because of the relatively short residual toxicity of Dylox in comparison with most of the other materials used in this test, adults emerging from pupae, or immigrating into the Dylox-treated plots from outside areas, were probably unharmed. With certain exceptions, Dylox applied at 3.6 ounces per acre caused only a slight reduction in numbers of most beneficial species, in comparison with results in untreated plots. The exceptions were the approximately 50% reduction in adults of *Orius* sp. and the virtual elimination of syrphid larvae with both dosages of Dylox.

TEST NO. 3.—On April 30, 1958, two dosages of Dylox and one of parathion were applied by aircraft on alfalfa in the Imperial Valley, California. The alfalfa was 24 inches tall, and 5 gallons of spray liquid were applied per acre. Each treatment was replicated three times, and each replicate was 135 feet wide and 1,294 feet long. The effects of the materials on the beneficial insects were evaluated 1 and 2 days after application (table 3).

The higher dosage of Dylox (8 ounces per acre) rather markedly reduced *H. convergens*. However, this spray was not quite so toxic as parathion applied at the same dosage. Adult syrphid and adults and nymphs of *N. ferus* were also markedly reduced by the 8-ounce dosage of Dylox. At 4 ounces per acre Dylox was not nearly so toxic to *H. convergens*. However, the lower dosage appeared to be as toxic as the higher dosage (8 ounces per acre) to syrphids and to *N. ferus*. In this test adults of *Orius* sp. were not affected to any extent by either Dylox dosage. Parathion applied at 8 ounces per acre was highly toxic to *H. convergens*, syrphids, and to *Orius* sp. and *N. ferus*.

TEST NO. 4.—This test was conducted for two reasons: (1) to ascertain the effect of Sevin® (*N*-methyl-1-naphthyl carbamate), Dylox, and heptachlor on numerous predaceous species, and (2) to follow population trends of the spotted alfalfa aphid in each of the treatments. Aphids were moderately abundant in the field when the test was initiated and heavy alate aphid migrations took place in the area during the testing period.

The sprays were applied by ground equipment on July 28, 1958, on alfalfa about 16 inches tall. Each treatment was replicated four times and each replicate was 60 feet wide and 120 feet long. The effects of the materials on the predaceous insects were evaluated 1, 2, 5, and 9 days after application (table 4). The initial mortality of the aphids in the plots at the time of spray application and the secondary effect of the materials on the resurgent aphid population were evaluated 2, 5, and 9 days after treatment (table 5).

The major part of the *H. convergens* population was in the larval stage. Parathion, the standard comparison material applied at 6.1 ounces per acre, essentially eliminated the lady beetle larvae but was not quite so toxic to

Table 3.—Effect of parathion and Dylox sprays[a] on predators in alfalfa in the Imperial Valley, California.

Treatment			No. of Predators per 300 Sweeps at Two Intervals (Days) After Treatment[b]									
			Hippodamia convergens		*Orius* sp.		*Chrysopa* spp.		Syrphids		*Nabis ferus*	
	Amount/Acre		1	2	1	2	1	2	1	2	1	2
Material	Ounces	Gal.	A–L	A–L	A–N	A–N	A–L	A–L	A	A	A–N	A–N
Parathion	8	5	14–11	11–3	7–16	34–41	1–2	0–0	7	19	0–0	2–1
Dylox	8	5	24–34	36–10	24–53	50–224	1–0	3–3	15	3	1–0	13–3
Dylox	4	5	39–136	56–115	19–37	50–191	4–0	6–1	17	3	1–0	6–9
Untreated	—	—	79–200	112–178	21–108	46–254	3–0	6–1	15	15	17–5	45–10

[a] Sprays applied to aircraft, April 30, 1958.
[b] A = adults; L = larvae; N = nymphs.

the adults. Parathion also caused moderate to severe reductions in adult and immature stages of *Orius* sp., *Geocoris* spp., *Chrysopa* spp., *N. ferus*, and in adults of *Collops vittatus* (Say). The toxicity of parathion to the entomophagous species mentioned above was generally comparable to results reported by van den Bosh *et al.* (1956), Stern & van den Bosch (1959), Stern *et al.* (1958), and Goodarzy & Davis (1958). On the ninth day after application adults of several entomophagous species invaded the plots from the surrounding untreated area and thus masked the initial kills by parathion. However, the effects of this material were still evident 9 days after application, as indicated by the general scarcity of immature forms in the plots.

Even though Sevin applied at 19 ounces per acre was not nearly so generally toxic as parathion, it markedly reduced the numbers of coccinellid adults and larvae. Adults and nymphs of *N. ferus* and adults of *C. vittatus* were also noticeably reduced.

Dylox applied at 9.5 ounces per acre destroyed about 30% of the coccinellid larvae. This material was highly toxic to adults of *Orius* sp., and even more toxic to the nymphs. It nearly eliminated the adults and nymphs of *N. ferus*.

Heptachlor applied at 2.4 ounces per acre destroyed about 66% of the adults and nymphs of *Geocoris* spp. and adults of *C. vittatus*. However, this low dosage of heptachlor, which is highly effective against larvae of both the alfalfa weevil, *Hypera postica* (Gyll.), (Anonymous, 1954), and the Egyptian alfalfa weevil, *H. brunneipennis* (Boh.) (Reynolds *et al.* 1955), was, of any treatment used in the experiments, by far the least toxic to the general entomophagous insect populations.

Sampling of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), 48 hours after treatment showed that parathion had given an apparent kill of only 66% of the apterous aphids. The Imperial Valley is one area in California where parathion has failed to give consistently good results (Stern & Reynolds 1958). However, in this instance, 6.1 ounces of parathion were applied per acre. Moreover, 48 hours after treatment only an occasional older nymph or mature apterous aphid was found on the alfalfa stems. Nearly all the apterous aphids in the sample were first-instar nymphs. They were either in small groups without a mature apterous aphid among them or the very small aphids were clustered about one of the invading alate aphids. Thus, initial kill of the incipient population with parathion had undoubtedly been much greater than 66%. However, as mentioned previously, parathion eliminated the aphid-feeding coccinellid population. Thus the invading alate aphids were essentially free to multiply unhindered.

As a result, the fifth day after application aphid populations appeared to be on the verge of an explosive increase in the parathion plots. If the experiment had been allowed to run its course, the authors believe it would

Table 4.—Relative toxicity of various insecticide sprays[a] to predators in alfalfa in the Imperial Valley, California.

Material and Ounces per Acre	No. of Predators per 100 Sweeps at Various Intervals (Days) After Treatment[b]											
	Hippodamia convergens				*Orius* sp.				*Geocoris* spp.			
	1	2	5	9	1	2	5	9	1	2	5	9
	A–L	A–L	A–L	A–L	A–N	A–N	A–N	A–N	A–N	A–N	A–N	A–N
Parathion, 6.1	56–28	55–148	77–17	389–13	172–38	336–40	701–11	2070–116	22–210	16–123	33–103	62–43
Sevin, 19	21–262	52–286	22–25	624–10	636–715	1064–869	981–324	2031–121	259–1263	129–1018	100–490	164–503
Dylox, 9.5	118–1758	156–2135	176–430	498–88	195–74	440–68	510–35	1177–244	322–1878	174–1420	95–613	367–1147
Heptachlor, 2.4	130–2874	148–3211	144–398	497–72	582–1124	900–1559	727–548	1891–603	113–558	81–628	10–39	46–100
Untreated	112–2996	125–3045	151–484	464–74	695–1156	840–1731	579–578	1154–328	362–1924	259–1775	83–490	319–979
	Chrysopa spp.				*Nabis ferus*				*Collops vittatus*[c]			
Parathion, 6.1	0–17	2–14	1–9	9–7	31–0	13–3	20–2	39–24	24	53	20	46
Sevin, 19	4–30	0–59	2–22	2–0	34–39	21–56	5–44	42–49	28	31	16	31
Dylox, 9.5	4–72	4–111	3–36	1–4	14–2	30–4	14–7	35–19	62	50	33	73
Heptachlor, 2.4	3–66	3–79	0–16	1–3	133–158	80–318	45–93	45–93	37	41	18	56
Untreated	12–81	1–86	3–32	4–0	158–247	84–338	19–127	42–181	105	113	27	54

[a] Applied by ground equipment, July 28, 1958.
[b] A = adults; L = larvae; N = nymphs.
[c] All A.

Table 5.—Population trends of the spotted alfalfa aphid following treatment with various insecticide sprays on alfalfa in the Imperial Valley, California, July 28, 1958.

Materials	Amount/Acre		No. of Apterous Aphids per 60 Alfalfa Stems on Days After Application Shown		
	Ounces	Gallons	2	5	9
Parathion	6.1	15	271	773	102
Sevin	19.0	12	510	498	140
Dylox	9.5	12	967	109	22
Heptachlor	2.4	12	689	81	23
Untreated	—	—	822	62	27

have showed clearly the need for increased frequency of treatment when the initial application also removed a significant biological check from the treated area. Unfortunately, however, on the eighth day after application a desert rainstorm struck in the plot area. An inch of rain fell in a very short period and washed most of the aphids from the plants. This washout masked the anticipated rapid resurgence of aphids in the parathion plots where the coccinellids had been eliminated.

A residue of older nymphs and apterous adults indicated that initial control of the incipient aphid population in the plots treated with Sevin was not quite so effective as with parathion. As in the parathion treatment, coccinellids were largely eliminated by Sevin but enough remained in the plots (approximately 3 per sweep) to prevent a buildup by surviving and invading aphids (unpublished data of K. S. Hagen, University of California, Department of Biological Control, Albany). Under other circumstances, where the initial coccinellid population was not so large, equally heavy coccinellid mortality combined with a large invasion of alate aphids could result in a rapid resurgence of the aphid.

Unpublished data of a junior author (Reynolds) indicate that Dylox at $\frac{1}{2}$ to $\frac{3}{4}$ pound per acre gives poor control of the spotted alfalfa aphid. A 30% mortality occurred in the coccinellid population where Dylox (9.5 ounces per acre) was applied. With tremendous numbers of alate aphids invading the plots, this reduction in lady beetles may account for the slight delay in coccinellids' eliminating the aphids in the Dylox plots as compared with the untreated plots where coccinellids effected a rather rapid cleanup of aphids.

Heptachlor applied at 2.4 ounces per acre has little effect on the aphid (unpublished data of the senior author). As mentioned previously, the coccinellids survived treatment with this material and thus were able to eliminate the incipient aphid population as well as the offspring produced by the invading alate aphids at about the same rate as in the untreated plots.

TEST NO. 5.—On August 25, 1958, various insecticide sprays were applied by aircraft on alfalfa at Dos Palos, California, to measure their effect on *Apanteles medicaginis* Muesebeck, the principal parasite of the alfalfa caterpillar, *Colias philodice eurytheme* Boisduval. Coccinellid adults present in the field in fair abundance were also counted. The alfalfa was 24 inches tall, and 8 gallons of liquid were applied per acre. Each treatment was replicated three times and each replicate plot was 175 feet wide and 1,660 feet long. The effects of the materials on the entomophagous species were evaluated 1 and 2 days after application.

Pretreatment counts were made of both *Apanteles medicaginis*, and *H. convergens* adults. These counts showed an average of 124 *A. medicaginis* and 99 cocinellid adults per 150 sweeps with a standard insect net. When the plots were sampled 1 day after treatment, the coccinellids and the parasite had dropped considerably in abundance in all treatments and in the untreated area. The data obtained suggests that Dylox at 8 and 4 ounces per acre and heptachlor and parathion at 2 ounces per acre may have little effect on *A. medicaginis*. Heptachlor also appeared to have little effect on the lady beetle population, while the 2-ounce dosage of parathion per acre was quite toxic to the lady beetles.

In this test, the highest dosage of Dylox (8 ounces per acre) caused a greater reduction in the coccinellid adults than in previous tests. One possible explanation may come from Metcalf and his coworkers (1959) who have showed that under alkaline conditions Dylox is rapidly converted to DDVP, which is closely related to Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical) in chemical properties. Phosdrin is highly toxic to cocinellids (Stern & van den Bosch 1959). Metcalf *et al.* (1959) have shown that at pH 8.0, the initial amount of DDVP builds up rapidly at a rate equal to the disappearance of Dylox. The concentration of DDVP reaches a maximum (55%) in about 2.5 hours, when only about 30% of the original Dylox is left.

It is probable, therefore, that in the field, when Dylox is mixed with slightly or moderately alkaline water and then allowed to remain in the nurse tank, the solution may well represent a mixture of Dylox plus DDVP. As a result, the selectivity otherwise characteristic of Dylox at low dosages could possibly be lost.

In the Dos Palos test about 20 minutes passed from the time of mixing the Dylox in water until the spray solution was applied to the field. This length of time may have been sufficient for a certain portion of the Dylox to convert to DDVP, which supposition, if correct, could account for the more toxic effect on the coccinellids than in other tests where the Dylox was mixed with the water and immediately sprayed on the test plots. Whether or not this was true in the case under discussion, the factor of rapid conversion of the somewhat selective Dylox to the broadly toxic DDVP must always be considered when Dylox is used in areas having alkaline waters.

DISCUSSION.—At 8 ounces or less per acre, Dylox is quite selective to a number of entomophagous insects which help maintain the general equilibrium position of certain field crop pests at a lower level than would exist in their absence. Above 8 ounces per acre, the selectivity of Dylox decreases. However, even at the higher dosages it has some desirable characteristics in comparison with certain compounds now in use to control field crop pests. Dylox is relatively nontoxic to honey bees, *Apis mellifera* L., (Anderson & Atkins 1958) and thus can be used to control numerous alfalfa seed pests when the seed fields are in bloom. The material also has a short toxic residue and thus emerging adult predators and parasites and

adults migrating into the field after treatment apear to be unharmed.

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Potential Use of Dylox and Other Insecticides in a Control Program for Field Crop Pests in California[1]

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ABSTRACT

Dylox® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) and several standard insecticides were evaluated for effectiveness on a rather wide range of insects damaging field crops in California. At varying dosages Dylox was effective in controlling the following pests on alfalfa hay: Alfalfa caterpillar, *Colias philodice eurytheme* Bdv.; webworm, *Loxostege* spp.; beet armyworm, *Laphygma exigua* (Hbn.); and variegated cutworm, *Peridroma margaritosa* (Haw.). On seed alfalfa Dylox gave high mortalities of lygus bugs, Say stink bug, *Chlorochroa sayi* Stål, and *Euschistus* sp. In addition to the above-mentioned pests on seed alfalfa, on cotton Dylox was effective on cotton leaf perforator, *Bucculatrix thurberiella* Busck, salt-marsh caterpillar, *Estigmene acrea* (Drury), and was promising for control of southern garden leafhopper, *Empoasca solana* DeL., and omniverous leafroller, *Platynota stultana* Wlshm. This chemical gave poor results when applied to cotton for control of cabbage looper, *Trichoplusia ni* (Hbn.), and bollworm, *Heliothis zea* (Boddie). In general, the residual period of toxic activity in the field lasted only a few days.

Dylox residues on alfalfa hay were below 1 p.p.m. 4 days after application at dosages of as much as 1 pound per acre. Only trace amounts were detected 10 days after application.

Dylox was no more effective than some of the other compounds evaluated. In view of the fact, however, that it is relatively nontoxic to honey bee, *Apis mellifera* L., populations and, depending upon dosage applied, less toxic to most entomophagous arthropods than many chemicals currently in use, Dylox appears to be a potentially useful compound for use on many California field crops.

In recent years numerous new insecticides have been evaluated in California for possible use against insects attacking field crops, particularly cotton, alfalfa grown for hay and seed, and sugar beets. Prior to incorporating a new compound into a chemical control program, which may thus result in intensive and widespread use, several factors must be considered in addition to the effect of the chemical on the injurious phytophagous insect species. Some of the most important considerations in the use of a new chemical are the residue at time of harvest, possible residue in milk when lactating animals are fed treated forage, mammalian toxicity, residual period of toxic activity, and effect on pollinating and entomophagous arthropod species.

Dylox®[4] (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) a relatively new compound, has been investigated and some data concerning the above aspects have been published. Robbins *et al.* (1956) found there was only a remote possibility of Dylox appearing in milk following oral administration of a large dose of P^{32}-labeled material to a lactating cow. Anderson & Atkins (1958) showed that Dylox was relatively nontoxic to honey bee, *Apis mellifera* L., populations which are of great importance in California agriculture.

Integrated control was defined by Stern *et al.* (1959) as pest control which combines the use of biological and chemical controls. Thus, when necessary, chemicals are

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[4] Known also as L13/59 and Dipterex. Produced by Farbenfabriken Bayer, Leverkusen, Germany, and furnished by Chemagro Corp., Kansas City, Missouri.

employed to suppress pest populations below the economic injury level, but, simultaneously, biological control agents are preserved at population levels of sufficient magnitude to maintain population regulation of the various pest species in the ecosystem to which the pest belongs. An important step toward integration is the development of selective insecticides. The paper by Stern *et al.* (1960) discusses the selective nature of Dylox. It was found that depending to some extent upon the amount of toxicant used per acre, Dylox was sufficiently selective that it might partially fulfill the requirements in an integrated control program. The present paper is concerned primarily with effect of Dylox on phytophagous species, residual period of toxic activity, and residue degradation on alfalfa hay. Following favorable preliminary field experiments and observations, research with Dylox was expanded to cover a rather wide range of insect pests. Where tests were repetitious, summarized numerical data are not presented, but a short discussion of results is given.

EFFECT OF DYLOX ON LEPIDOPTEROUS LARVAE ATTACKING ALFALFA HAY.—The larvae of several lepidopterous insects at times severely damage alfalfa hay in California. The most important of these pests are the alfalfa caterpillar, *Colias philodice eurytheme* Bdv.; beet armyworm, *Laphygma exigua* (Hbn.); western yellow-striped armyworm, *Prodenia praefica* Grote; variegated cutworm, *Peridroma margaritosa* (Haw.); and webworms, *Loxostege* spp.

Experiment No. 1.—The alfalfa caterpillar is the most important of the lepidopterous larvae attacking alfalfa in California. At times it may completely defoliate alfalfa in the commercial hay-growing regions of the warm interior valleys. Damage is usually confined to late June through September.

On October 7, 1955, several insecticides were tested on a damaging infestation of the alfalfa caterpillar in the Antelope Valley near Lancaster, California. The infestation in the experimental field averaged about 100 larvae per 180° sweep with a standard insect net. Insecticides were applied as sprays at a pressure of 60 p.s.i. with a ground sprayer equipped with a boom 20 feet wide. Twelve hollow-cone nozzles were arranged on the boom for broadcast spraying. Each treatment was replicated three times, each replicate being 20 feet wide and 300 feet long. The treatments were evaluated 3 days after application by making 10 individual sweeps with a standard insect net in each replicate or 30 sweeps per treatment. The data are summarized in table 1.

Table 1.—Results of insecticide sprays applied October 7, 1955 on alfalfa hay for control of *Colias philodice eurytheme* near Lancaster, California.

MATERIAL	AMOUNT PER ACRE		NO. OF LARVAE PER 30 SWEEPS OF NET 3 DAYS AFTER APPLICATION
	Gallons	Toxicant (lb.)	
Heptachlor	10	0.3	1,025
Dylox	11.5	1.2	2
Dylox	11	0.6	4
Dylox	11.5	0.3	7
Parathion	8	0.2	21
Untreated	—	—	4,260
L.S.D. at 5% level			564

Table 2.—Results of two insecticide spray applications made for control of alfalfa caterpillar, beet armyworm, and alfalfa webworm on alfalfa hay in the Imperial Valley, California.

MATERIAL	AMOUNT/ACRE		NO. ALFALFA CATERPILLAR[a]		NO. BEET ARMYWORM LARVAE[a]		NO. ALFALFA WEBWORM LARVAE[a]
	Gallons	Oz. Toxicant	Sept. 21	Sept. 23	Sept. 21	Sept. 23	SEPT. 23
Field No. 1—Treated September 19, 1957							
Dylox	5	4.0	21	2			
Phosdrin	5	1.5	12	1			
Check	—	—	1,495	505[b]			
Field No. 2—Treated September 20, 1957							
Dylox	5	4.0	213	49	517	199	14
Phosdrin	5	1.5	767	566	1,955	478	[c]
Check	—	—	6,271	1,975[b]	3,214	585[b]	818

[a] Per 300 net sweeps.
[b] Reduction in untreated population owing to pupation.
[c] Reduction of alfalfa webworm with Phosdrin was estimated to be no better than 20%.

Dylox applied at 1.2, 0.6 and 0.3 pound per acre all gave excellent control of the alfalfa caterpillar. Parathion applied at 0.2 pound per acre was used as a standard for comparison and it gave a kill nearly comparable to each of the three dosages of Dylox. Heptachlor applied at 0.3 pound per acre reduced the larval population by approximately 75%. However, this degree of kill was not sufficient since the remaining population of 34 larvae per sweep 3 days after treatment was still well above the economic threshold.

Experiment No. 2.—For this experiment two alfalfa fields were treated on September 19 and 20, 1957, to study the effects of Dylox and Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical) on lepidopterous larvae attacking alfalfa. In the first (September 19) the two materials were applied to a field with a subeconomic alfalfa caterpillar population. In the second (September 20), the two materials were applied on alfalfa infested with a complex of lepidopterous larvae consisting of the alfalfa caterpillar, beet armyworm, and the alfalfa webworm, *L. commixtalis* (Wlk.).

In both cases the sprays were applied by airplane. The plots were 120 feet wide and 1,240 feet long and each treatment was replicated three times. In the first field the effects of the materials were evaluated 1 and 4 days after application and in the second the effect of the materials was evaluated 1 and 3 days after treatment. In each case 10 samples of 10 sweeps each were taken in each replicate using a standard sweep net or a total of 300 sweeps per replicate. The data are summarized in table 2.

Dylox applied at 4 ounces per acre very satisfactorily reduced the alfalfa caterpillar and alfalfa webworm infestation, while approximately 84% of the beet armyworms were eliminated. This result plus results of tests on other crops would seem to indicate that dosages of Dylox somewhat greater than 4 ounces per acre may be necessary for satisfactory control of beet armyworm. Phosdrin, when used at the low dosage of 1.5 ounces per acre, reduced the infestation of the alfalfa caterpillar well below damaging abundance (10 nonparasitized larvae per sweep) but this dosage did not give adequate control of the beet armyworm and alfalfa webworm.

In a miscellaneous experiment conducted in the Imperial Valley, 4 ounces of Dylox per acre gave 90%, 66%, and 98% control of the alfalfa caterpillar, beet armyworm,

Table 3.—Results of several insecticide sprays applied by airplane for control of *Peridroma margaritosa* on alfalfa hay.

Materials	Toxicant (Lb. per Acre)	Larvae per 25 Sweeps of Net, May 25
Field No. 1—treated on May 22, 1956		
Dylox	1.0	19
Parathion	0.5	85
Heptachlor	0.5	140
Phosdrin[a]	0.3	202
Trithion	1.0	309
Check	—	535

		Larvae per 300 Sweeps of Net	
Field No. 2—treated on April 30, 1958		May 1	May 3
Dylox	0.5	43	32
Dylox	0.3	128	87
Parathion	0.5	75	44
Check	—	195	87

[a] Treatments in Field No. 1 were applied in the morning. In view of the short residual nature of Phosdrin and the nocturnal feeding habits of the larvae, it is quite possible that much of the toxicity of Phosdrin had dissipated prior to the movement of the larvae onto the plants to feed during the evening.

and alfalfa webworm, respectively, 18 hours after treatment, while 72 hours later the control was 98%, 60%, and 90%, respectively, for these same insects. The Dylox was applied with a ground sprayer and each treatment was replicated four times. In a second miscellaneous experiment conducted in the San Joaquin Valley, 8 and 4 ounces of Dylox per acre gave 99% and 92% control, respectively, of the alfalfa caterpillar 24 hours after treatment; 72 hours later reductions with both dosages were above 97%. In this case the sprays were applied by airplane and treatments were replicated three times.

Experiment No. 3.—This involved the variegated cutworm, *Peridroma margaritosa*, which is occasionally damaging to alfalfa hay during the spring in California. The larvae damage alfalfa in three ways: by destroying the new shoots in seedling stands; by preventing regrowth following cutting; and by defoliating the large stems. In general, chemical control of this species has not been entirely satisfactory. The principal difficulty lies in the habits of the larvae which characteristically hide in the trash at the base of the alfalfa plants during the day and generally move onto the plants to feed at night. For this reason evening applications of insecticide give better control. However, nighttime observations indicated that not all the larvae move onto the plant during a given night. Some apparently remain under the trash at the base of the plants for one or more nights. Occasionally the majority of the larvae remain in the litter at night. For these reasons the uniform elimination of larval populations of this species with insecticides is difficult.

During the course of the investigations on the variegated cutworm two fields were used to test the effects of various chemical compounds on the larvae. Materials in both fields were applied as sprays by airplane at the rate of 5 gallons of liquid per acre. The first was treated in the morning near Lancaster in the Mojave desert on May 22, 1956. In this case 5-acre, nonreplicated blocks were used. The second was treated in the evening of April 30, 1958 in the Imperial Valley. In this case each treatment was replicated three times. Each replicate was 90 feet wide and 1,294 feet long. The data from the two tests are summarized in table 3.

Results in the first field (table 3) show that Dylox applied at 1.0 pound per acre gave excellent control of *P. margaritosa*. In the second field 0.3 pound was unsatisfactory while 0.5 pound was moderately effective. Parathion applied at 0.5 pound per acre, the currently recommended dosage in California for this cutworm on alfalfa, was not so effective as the equivalent dosage of Dylox. On the basis of this experiment, 0.75 to 1.0 pound Dylox per acre is probably warranted. Heptachlor, Phosdrin and Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) applied at 0.5, 0.3, and 1.0 pound per acre, respectively, were unsatisfactory.

Effect of Dylox on Insect Pests of Alfalfa Seed.—*Lygus* spp. are probably the most important insect pests of alfalfa seed in California. Several stink bug species are also extremely damaging on occasion. Included in this group are *Chlorochroa sayi* Stål and *Euschistus* sp. (probably *E. conspersus* Uhler).

Experiments Nos. 4 and 5.—These experiments involved *Lygus* spp., which have become increasingly difficult to kill with such chlorinated-hydrocarbon insecticides as toxaphene and DDT in the interior valleys of California. This tolerance of lygus bugs for these insecticides becomes more noticeable as the seed-production season advances. Consequently, it often has been necessary to substitute organophosphorous insecticides such as malathion or parathion, which create considerable hazard to honey bee populations.

In the present investigations several experiments involving both ground and aerial application of insecticides were conducted for the control of lygus bugs. The data from two of these are summarized in tables 4 and 5.

In experiment No. 4 (table 4), applications were made with a ground sprayer at a pressure of 60 p.s.i. and the boom was equipped with hollow-cone nozzles arranged 18 inches apart for broadcast spraying. Each treatment was replicated twice and each replicate was 133 feet wide and 600 feet long.

In experiment No. 5 (table 5), each treatment consisted of a large nonreplicated 10-acre block. A check plot of comparable size adjoined each treatment. In this case, materials were applied by airplane at the rate of 5 gallons of spray per acre.

In experiment No. 4 (table 4), Dylox applied at 12.8

Table 4.—Results obtained with insecticides applied June 14, 1957 as sprays by ground equipment to seed alfalfa for control of lygus bugs. Hemet, California.

Materials	Amount/Acre		Lygus Bugs per 50 Net Sweeps[a]							
	Gallons	Oz. Toxicant	June 15		June 17		June 20		June 24	
			A	N	A	N	A	N	A	N
Dylox	8.0	12.8	3	2	30	0	267	6	235	234
Phosdrin	6.8	4.3	1	7	35	3	178	15	116	130
DDT	8.0	18.7	2	7	16	2	113	2	147	8
Thiodan	8.0	12.8	2	6	28	0	198	0	147	15
Check	—	—	414	436	293	150	Treated prior to June 20			

[a] A, adults; N, nymphs. Because of heavy lygus bug infested area around plots and because of check strips through plots, treated areas were subject to a rapid reinvasion of adult lygus bugs. Consequently, after first counts, adult populations mean very little.

ounces per acre produced an excellent kill of lygus bugs 1 day after application. However, the treated area was reinvaded by adult lygus bugs between the first and third day after application. A strong wind started on the second day after treatment and persisted through the third day. Thus, the numbers of lygus bugs sampled were reduced. The nymphs remained at a low level in the Dylox plots through the sixth day after application and increased sharply when the plots were sampled 10 days after treatment, whereas the invading adults increased to high numbers by the sixth day after application. It appears that initial kill of lygus in both experiments was satisfactory 1 day after application but the residual period of activity was but a few days. The biological data from the field are supported by the laboratory residue analysis in table 9.

Phosdrin, DDT, and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) applied at 4.3, 18.7 and 12.8 ounces per acre, respectively, also gave satisfactory control of lygus nymphs and adults 1 day after treatment. However, the reinvading adults obscured the effectiveness of these materials by the sixth day after application. DDT, on the other hand, showed moderate residual toxicity against both adults and nymphs. Thiodan appeared to have a similar action to DDT while Phosdrin with its short residual action was somewhat comparable to Dylox.

In experiment No. 5 (table 5), Dylox applied at 1.0 pound (16 ounces) per acre generally was comparable to Dylox at 12.8 ounces per acre in experiment No. 4. The data from these two tests plus those from additional tests on cotton indicate that Dylox, at rates of 0.75 to 1.0 pound per acre, gives satisfactory control of lygus bugs. However, where the treated fields are subjected to rapid reinvasion and heavy nymphal production is occurring, the material may not be quite so satisfactory as DDT or other materials with a long residual action.

Experiment No. 6.—This experiment involved two stink bug species. These were Say stink bug, *Chlorochroa sayi* Stål, and *Euschistus* sp. (probably *E. conspersus*). Of the two, Say stink bug is generally considered much the easier to kill. In the three fields in which tests were conducted against these insects, applications were made as aerial sprays applied at the rate of 5 gallons of liquid per acre. In field No. 1 each treatment was replicated three times, each replicate being 120 feet wide and 1,200 feet long. In field No. 2, each treatment was replicated twice and each replicate was 180 feet wide and 1,200 feet long. In field No. 3, each treatment was applied to a 5-acre nonreplicated block. Treatments were evaluated by sweeping with a standard insect net. The data obtained from the three fields in experiment No. 6 are summarized in table 6.

Table 5.—Control of lygus bugs with insecticides applied July 10, 1957 as sprays by airplane to seed alfalfa. Imperial Valley, California.

Material[a]	Toxicant (Lb. per Acre)	Lygus Bugs/15 Net Sweeps			
		1 Day		7 Days	
		Adults	Nymphs	Adults	Nymphs
Dylox	1.0	11	4	11	27
Check	—	202	234	—[b]	—
Malathion	0.75	0	2	21	96
Check	—	182	334	—[b]	—

[a] Each treatment was a 10-acre nonreplicated block with an adjoining check area of comparable size.
[b] Checks treated prior to 7-day count.

Table 6.—Control of stink bugs in three experiments with insecticides applied as sprays with airplane on seed alfalfa. Imperial Valley, California.

Materials	Toxicant (Lb. per Acre)	Total Stink Bugs/100 Net Sweeps		
Field No. 1, Applied May 9, 1958 (Chlorochroa sayi)[a]				
		1 Day[b]	*3 Days*	*13 Days*
Dylox	0.5	0	0	14
Dylox	1.0	0	0	15
Methyl parathion	0.4	0	0	17
Field No. 2, Applied July 10, 1957 (Euschistus sp.)[c]				
		1 Day		*2 Days*
Parathion	0.5	25		26
Dylox	1.5	18		39
Phosdrin	0.5	19		73
Check	—	96		261
Field No. 3, Applied June 4, 1956 (Euschistus sp.)[c]				
Dylox	1.0	38		10
Phosdrin	0.65	30		27
Check	—	127		Treated

[a] High population owing to migration into field from surrounding desert.
[b] Pretreatment count averaged 362 stink bugs per 100 sweeps of net.
[c] Probably *E. conspersus*.

In the first field which involved the Say stink bug, Dylox was applied at 0.5 and 1.0 pound per acre. Both dosages gave excellent kills of adults and nymphs of *C. sayi*. Methyl parathion applied at 0.4 pound per acre gave comparable results. However, of the two materials, Dylox must be considered the more desirable because of its relatively low toxicity to honey bees. A small number of *C. sayi* nymphs were swept from all the treated plots after 13 days. These apparently hatched from egg masses deposited prior to the treatment. Evidently, as with lygus bugs, initial control of Say stink bug with Dylox was excellent but the material did not persist to kill the hatching nymphs.

In the second and third fields where *Euschistus* sp. was involved, Dylox was applied at 1.0 and 1.5 pounds per acre. Control was not nearly so satisfactory as in field No. 1 where the Say stink bug was the species of concern. However, as mentioned above, *Euschistus* sp. is a difficult pest to control and commercial materials are at best only moderately satisfactory in the areas where this experiment was conducted. Parathion and methyl parathion are the two materials most commonly used to control this pest on alfalfa seed crops and results of this experiment indicate that Dylox at 1.0 to 1.5 pounds per acre gives a comparable reduction. Moreover, Dylox has the advantage of being relatively nontoxic to honey bees. Phosdrin applied at approximately 0.5 pound per acre gave initial kills comparable to parathion and Dylox, but apparently because of its very short residue, results in general have not been so satisfactory where stink bugs were migrating into the test plots.

EFFECT OF DYLOX ON CERTAIN INSECT PESTS OF COTTON.—Certain lepidopterous and hemipterous insects rank among the most serious pests of cotton in California.

Table 7. Control of *Bucculatrix thurberiella* with several insecticides applied June 11, 1956 as sprays by airplane on stub cotton. Imperial Valley, California.

Materials[a]	Toxicant (Lb. per Acre)	No. Larvae per 50 Leaves[b]		
		June 12	June 18[c]	June 22
Dylox	1.0	0	4	39
Trithion	1.0	0	1	4
Guthion	0.75	0	3	16
Malathion	1.0	0	1	20
Parathion	0.5	0	0	83

[a] Applied at the rate of 5 gallons per acre.
[b] Pretreatment count (June 11) averaged 6.7 larvae per leaf. Because of imminent defoliation by this high population if left untreated, no untreated check areas were left.
[c] All small larvae.

In the hemipterous group lygus and stink bugs can be, and often are, extremely damaging. Numerous tests were conducted in cotton fields to test the effect of Dylox on these two pests. As on alfalfa seed 1 pound of Dylox per acre generally gave sufficient control of lygus and stink bugs in cotton, and for this reason the data obtained from the studies on cotton with these two pests are omitted.

Experiment No. 7.—The cotton leaf perforator, *Bucculatrix thurberiella* Busck, is one of the most damaging pests of cotton in southern California. If the cotton fields are not frequently surveyed for this species and applications timed properly, populations may increase with such rapidity that fields may be completely defoliated before control measures can be applied. Prior to 1956 the chlorinated-hydrocarbon insecticides were effective for control. Since then it has become necessary to use organophosphorous insecticides. Parathion or malathion are the materials most commonly used. However, secondary pest infestations, particularly bollworms (*Heliothus zea* (Boddie)), often increase after use of these materials. These outbreaks apparently result from reductions in the beneficial insect populations. In addition to destroying many of the entomophagous species, parathion and malathion are relatively ineffective on certain phytophagous species which reproduce relatively freely when their natural enemies are eliminated.

From 1956 to 1958 numerous experiments were conducted to test the effect of compounds on the cotton leaf perforator. Results of one of these tests are presented here. However, the data are typical of those obtained in the others. In this experimental field the cotton leaf perforator larvae were extremely abundant and threatened to defoliate the field within a short period. Numerous insecticides were applied by airplane at the rate of 5 gallons of liquid per acre. Because of the extremely threatening nature of the infestation and large plot size (5 acres), no area was left untreated. However, a pretreatment count revealed a population averaging 6.7 leaf perforator larvae per leaf. The data obtained in this experiment are summarized in table 7.

All treatments reduced the population from 6.7 larvae per leaf to 0 per leaf in 24 hours. Trithion at 1.0 pound per acre gave the best residual period of control. There was little difference between the other materials.

Observations in commercially treated cotton fields subsequent to the above described experiment have revealed that Dylox at 0.75 pound per acre gives excellent control of *B. thurberiella* and that a dosage of 0.5 pound per acre warrants further investigation.

Experiment No. 8.—Larvae of the salt-marsh caterpillar, *Estigmene acrea* (Drury), often become extremely abundant late in the cotton growing season and may prematurely defoliate the plants. The species is difficult to kill with insecticides, and resistance to chlorinated-hydrocarbon materials is suspected in several areas of the Southwestern United States. In 1,955 of the current investigations very high mortalities were obtained with Dylox; interestingly, complete kill took place almost immediately after application. Similarly, rapid kills by Dylox have been observed in related Arctiid species. More recently, however, kills of the salt-marsh caterpillar have been neither so rapid nor the rate of mortality so complete as in the earlier experiments. Nevertheless, Dylox at 1.0 to 1.5 pounds per acre still appeared to be an excellent compound for control of this pest.

The two fields (table 8) were treated by airplane using 5 gallons of liquid per acre. Each treatment was replicated twice on 5-acre blocks about one-fourth mile long. In each case samples were taken from each end of the two replicates to give a total of four samples from each treatment. Sampling consisted of counting all larvae on the top 12-inch terminal of 100 plants per treatment. The data are summarized in table 8.

Dylox at 1.0 to 1.5 pounds per acre was not so effective in the two fields composing this experiment as in earlier trials. However, control was adequate at the higher dosages. At 0.5 pound per acre (field No. 1) control was unsatisfactory.

Effects of Dylox on Miscellaneous Cotton Pests.—In addition to its favorable performance against lygus bugs, stink bugs, cotton leaf perforator, and salt-marsh caterpillar, Dylox, at the rate of 1.0 pound per acre, has shown promise as a control for the southern garden leafhopper, *Empoasca solana* DeL., and the

Table 8.—Control of *Estigmene acrea* on cotton with Dylox and Dilan. Palo Verde Valley, California.

Materials[a]	Ounces Toxicant per Acre	October 11—No. Larvae per 100 12-Inch Plant Terminals		
		Medium	Large	
Field No. 1—Treated October 7, 1957				
Dylox	7	4	52	
	11	4	10	
	19	4	6	
Dilan[b]	16	4	7	
Check	—	10	48	
Field No. 2—Treated October 8, 1957[c]		October 11—No. Larvae per 100 12-Inch Plant Terminals		
		Small	Medium	Large
Dylox	8	8	22	56
	16	0	7	42
	24	0	5	39
Check	—	20	60	129

[a] Materials applied by airplane as sprays at the rate of 5 gallons per acre.
[b] 1 part 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts 1,1-bis(*p*-chlorophenyl)-2-nitrobutane.
[c] Field No. 2. was a plant-4-skip-4-row planting. In an attempt to conserve material, the pilot plugged some center nozzles in the spray boom and flew over the skipped area, spraying the four rows on each side. Thus, only every other skipped area was flown. This procedure unquestionably reduced somewhat the effectiveness of these treatments.

omniverous leafroller, *Platynota stultana* Wlshm. In the case of *E. solana* testing at lower dosages appears warranted. This compound is unsatisfactory in control of cabbage looper, *Trichoplusia ni* (Hbn.). Properly timed applications, 1.0 to 1.5 pounds of Dylox per acre have given some control of small cotton bollworms, but at these dosages the material has not been so consistently effective as DDT at 1.5 to 2.0 pounds per acre.

DYLOX RESIDUES ON ALFALFA HAY.—Three field applications were made in which the persistence of Dylox residues in alfalfa hay was determined. Dosages of Dylox ranged from about 0.25 to 1.0 pound per acre. Applications were made with a ground sprayer equipped with a 30-foot boom. Hollow cone nozzles were located at 18-inch intervals along the boom. Sprays were applied at a pressure of 60 p.s.i., and approximately 10 gallons of liquid were delivered per acre. Each treatment was replicated four times.

The sprayed and check (unsprayed) alfalfa were sampled by cutting alfalfa stems about 2 inches above ground level.

Dylox residues were determined by the electrometric cholinesterase procedure of Hensel *et al.* (1954), briefly described as follows. Fresh samples of alfalfa were run through a bread slicer and then quartered down to a 100-gm. subsample. The subsample was ground in a Waring Blendor with 300 ml. of water for 3 minutes and the homogenate filtered through three layers of cheesecloth squeezed dry. The volume of filtrate consistently totaled 275 to 280 ml. The pH of the filtrate was adjusted to 7.0 by adding a few drops of aqueous potassium hydroxide and the volume adjusted to 300 ml. A 35-ml. aliquot was placed in a 50-ml. volumetric flask, 5 ml. of human blood plasma added and the total volume was made up to 50 ml. with neutral water. (In these calculations it was assumed that the weight of alfalfa in 35 ml. of filtrate is simply $(35/300)(100 \text{ gm.}) = 11.7 \text{ gm.}$) The flask was then kept at 37.5° C. for 70 minutes. A 1-ml. aliquot was then placed in a test tube along with 1 ml. of Veronal buffer (pH 8.00), kept at 37.5° C. for 10 minutes, and the pH measured. A 0.2-ml. solution of 0.66 M acetylcholine bromide was then added to the test tube, the mixture kept at 37.5° C. for 2 hours and the final pH measured. The per cent inhibition was calculated in the usual manner.

Standard curves were obtained by adding calculated amounts of Dylox to 35 ml. of filtrate obtained from untreated alfalfa-water homogenate and diluted to volume in 50-ml. flasks. The results are shown in table 9. In experiment No. 1, the residues at 0 and 1 day are not accurate, as the values were beyond the range of the standard curve. In the subsequent two tests the 0- and 1-day samples were diluted and the results corrected, in order that samples on these dates would remain within the limits of the standard curve. In experiment No. 2, owing to an error, the entire alfalfa stem was not taken for analysis on the 0- and 1-day sample; only the upper portion was sampled. Consequently, the residues shown on these dates are higher than they actually were. As a result, only in experiment No. 3 do the 0- and 1-day samples show accurate residues.

Based on the data obtained in these three experiments, it does not appear that excessive residues will be a problem within approximately 7 days after application even at rates of Dylox as high as 1.0 pound per acre—a dosage somewhat in excess of those contemplated for use on alfalfa hay in California.

Table 9.—Residues of Dylox persisting on alfalfa hay following spray application by ground equipment.

OUNCES DYLOX PER ACRE	RESIDUE ON INDICATED DAYS AFTER APPLICATION (P.P.M.)					
	Alfalfa about 12 inches tall—Treated July 23, 1956					
	0[a]	1[a]	2	4	8	16
17	2.70	2.16	1.28	0.26	0.03	0.00
	Alfalfa about 12 inches tall—Treated Oct. 8, 1957					
	0[b]	1[b]	3	6	10	14
3.7	>70	4.9	0.20	<0.1	—	—
6.9	>70	9.6	0.74	0.2	<0.1	—
12.6	>70	24.1	2.20	0.3	0.15	<0.1
	Alfalfa about 12 inches tall—Treated June 16, 1958					
	0	1	2	4	7	10
8.0	9.05	6.30	2.53	0.415	<0.09	—
16.0	>11.20	11.00	3.75	0.813	0.114	<0.09

[a] Actual residues higher than indicated as residues were beyond range of standard curve.

[b] Actual residues lower than indicated owing to error in sample preparation for analysis.

DISCUSSION.—In general, Dylox is no more effective for control of many damaging insects than several other compounds currently in use on cotton and other field crops in California. However, in view of several desirable characteristics of this compound and because insecticide resistance in several areas of the State has necessitated use of organophosphorous compounds, Dylox appears to be a potentially useful chemical at the present time. Dylox is considered most effective when ingested; consequently, in most cases lower dosages are effective for lepidopterous larvae than for sucking species such as lygus and stink bugs. Usually effective dosages on alfalfa hay are somewhat lower than on many other crops, such as cotton, at least in midseason when the cotton plants are large. This appears to be true with Dylox as well.

Stern *et al.* (1960) reported that at dosages of 4 to 8 ounces of toxicant per acre, Dylox was not so detrimental to beneficial insects as several chemicals recommended for use on alfalfa hay. This may prove to be of great value in the case of the spotted alfalfa aphid, *Therioaphis maculata* Buckton, on this crop. Although Dylox is only slightly toxic to this aphid, its use for control of several species of lepidopterous larvae may spare the most important predators and parasites in numbers sufficient to regulate the aphid population.

As honey bee populations are not decimated by the use of Dylox on alfalfa during seed production, considerable use of this chemical may be anticipated. To be effective for control of lygus and stink bugs, dosages in excess of those considered somewhat selective are necessary. Nevertheless, it should have value for control of lepidopterous larvae at selective dosages and should prove useful particularly for control of stink bugs and perhaps to a lesser extent for lygus bugs at higher, less selective dosages.

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Salt-Marsh Caterpillar Control on Cotton in Arizona[1]

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ABSTRACT

In 1955 tests at Yuma, mixtures of toxaphene and DDT, formulated both as dusts and sprays, effectively controlled the salt-marsh caterpillar, *Estigmene acrea* (Drury); these mixtures were slightly more effective than parathion formulations and much more effective than formulations of malathion, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) and sodium fluosilicate. A spray formulation containing methyl parathion and toxaphene also gave satisfactory control.

Results of 1958 tests near Phoenix indicated an apparent resistance to toxaphene-DDT mixtures although good control was obtained with formulations containing Dilan (1 part 1,-1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts 1,1-bis(*p*-chlorophenyl)-2-nitrobutane), Dylox® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), parathion, Sevin® (1-naphthyl-*N*-methyl carbamate), and a mixture of endrin plus methyl parathion.

The salt-marsh caterpillar, *Estigmene acrea* (Drury), is considered a major pest of cotton in Arizona during September and October. Prior to 1957, effective control was obtained with a dust mixture of 15% toxaphene plus 5% DDT or its spray equivalent (Stevenson *et al.* 1957). Stevenson *et al.* (1957) suspected that salt-marsh caterpillars were becoming resistant to the toxaphene-DDT mixtures but reported that parathion and Dilan (1 part 1,-1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts 1,-1-bis(*p*-chlorophenyl)-2-nitrobutane) were effective. In cage tests, in Texas, Bottger *et al.* (1958) obtained excellent control with Sevin® (1 naphthyl-*N*-methyl carbamate) sprays applied at the rate of 2 pounds of toxicant per acre.

A number of commonly used insecticides were evaluated in 1955 for control of salt-marsh caterpillars near Yuma. Since 1955, the salt-marsh caterpillar has become increasingly difficult to control with recommended toxaphene-DDT mixtures in the cotton-growing districts of southern Arizona. Owing to widespread and repeated failures under practical field conditions, growers have largely abandoned toxaphene-DDT mixtures in favor of parathion applications, ordinarily of a 2% dust or its equivalent. Experiments were therefore conducted in the Phoenix area in 1958 to evaluate the comparative effectiveness of parathion, toxaphene-DDT mixtures and other insecticides against the salt-marsh caterpillar.

METHODS.—All insecticides were applied by aircraft in accordance with common grower practice. In 1955 tests at Yuma, treatments in two experiments (tables 1 and 2) were applied to single plots of five acres each. In a third, experiment plots were 0.6 acre in area, with treatments replicated four times (table 3). Sprays were applied at the rates shown in the tables in 8 gallons of water per acre. In the first experiment, 100 plants were examined and the number of surviving caterpillars (primarily second- and third-instar larvae) counted 3 and 6 days after treatments. In the second and third experiments caterpillars were larger and could be more easily seen. Infestation data were therefore obtained by having three people in different locations of each plot count the number of visible larvae on a row of cotton in a 2-minute period.

Table 1.—Effectiveness of airplane sprays in controlling the salt-marsh caterpillar, Yuma, Arizona, 1955.

Toxicant and Pounds per Acre (Emulsion Concentrates Used)	Larvae per 100 Plants After 3 Days		Larvae per 100 Plants After 6 Days	
	No.	Per Cent Control	No.	Per Cent Control
Diazinon, 0.5	52	51	89	25
Diazinon, 0.5+Toxaphene, 1.5	31	71	63	47
Methyl parathion, 0.5 plus Toxaphene, 1.5	24	78	27	77
Untreated	109	—	118	—

Table 2.—Effectiveness of airplane applications of various insecticides in controlling the salt-marsh caterpillar, Yuma, Arizona, 1955.

Toxicant and Pounds per Acre	Larvae Observed in a 2-Minute Period After 4 Days		Larvae Observed in a 2-Minute Period After 9 Days	
	No.	Per Cent Control	No.	Per Cent Control
Sprays (Emulsion Concentrates Used):				
Endrin, 0.4	18.8	67	25.8	25
Chlorothion, 0.25	52.9	7	23.0	33
Malathion, 1.25	24.3	57	10.1	71
Dusts:				
10% Malathion, 25	37.5	34	20.3	41
5% DDT+15% toxaphene +40% sulfur, 30	5.0	91	0.4	99
5% DDT+15% toxaphene +40% sulfur, 40	0.5	99	0.0	100
2% Parathion, 25	20.6	64	4.4	87
Untreated	56.9	—	34.3	—

In 1958 tests in the Phoenix area, treatments were ap-

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plied in the first two experiments (tables 4 and 5) against newly hatched larvae, 1 day old or less. Four days after treatment data were taken from 30 infested plants from each plot by counting the surviving larvae on the most heavily infested leaf from each plant. In the remaining two 1958 experiments (tables 6 and 7) treatments were applied against third- to fifth-instar larvae, no longer in the "cluster stage." Data were based on counts of larvae present on the terminal 12 inches of 50 plants selected at random in each plot. In all tables "per cent control" refers to the percentage reduction of infestations in treated plots as compared with infestations observed the same day in untreated plots.

1955 Experiments (Yuma): The data in table 1 indicate that commercial control of the salt-marsh caterpillar was obtained with a spray formulation containing 0.5 lb. methyl parathion and 1.5 lbs. toxaphene per acre. Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) at 0.5 lb. per acre was ineffective although the degree of control was increased when 1.5 lbs. of toxaphene per acre was added.

The data in table 2 indicate that toxaphene-DDT dust mixtures were very effective in 1955 tests, that control was not greatly improved when poundages per acre were increased from 30 to 40 and that no control advantage resulted when a 2% parathion dust was used. In this test spray formulations were less effective than dusts.

The data in table 3 indicate that in 1955 a dust formulation of toxaphene-DDT was more effective than spray formulations of these toxicants and that no advantage was gained from adding parathion to such mixtures. Sodium fluosilicate, in a dust formulation, gave inadequate control.

1958 Experiments (Phoenix Area): The data in table 4 show that the commonly used toxaphene-DDT mixture gave only 48% control of first-instar larvae while formulations of Dilan and parathion reduced infestations 97% and 99%, respectively. By excessively increasing toxaphene and DDT dosages to 6 lbs. and 3 lbs. per acre, respectively (table 5), the effectiveness of this combination was increased. In an experiment where 12 weekly applications of 15% toxaphene-5% DDT dust were applied, the larval populations were reduced only 50%, and 40% of the foliage had been consumed at the time of the final count (table 7). This 1958 failure is in decided contrast to the high degree of control obtained in 1955 with single treatments (tables 2 and 3) and substantiates observations of Stevenson *et al.* (1957) regarding the apparent resistance of the salt-marsh caterpillar to toxaphene-DDT mixtures.

Spray applications of Dilan were most effective against the clustered, younger larvae of the first two instars (tables 4 and 5) and provided good residual control, but were less effective against larvae of the third-instar or older (table 6).

Effective control of larvae of all stages was obtained with formulations of Dylox® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) (table 6), parathion (table 6), Sevin (tables 6 and 7) and a dust mixture containing 1.5% endrin plus 1.5% methyl parathion. A 10%

Table 3.—Effectiveness of airplane applications of various insecticides in controlling the salt-marsh caterpillar, Yuma, Arizona, 1955.

Toxicant and Pounds per Acre	Larvae Observed in a 2-Minute Period			
	3 Days		6 Days	
	No.	Per Cent Control	No.	Per Cent Control
Sprays: (Emulsion Concentrates Used)				
Toxaphene, 2+DDT, 1	23.4	45	15.8	58
Toxaphene, 4+DDT, 1	10.3	76	5.0	87
Parathion, 0.5+toxaphene, 2+DDT, 1	19.6	54	18.6	50
Parathion, 1.0+toxaphene, 4+DDT, 2	11.5	73	12.9	66
Dusts:				
15% toxaphene+5% DDT +40% sulfur, 40	3.5	92	5.2	86
2% Parathion+10% DDT +50% sulfur, 30	2.5	74	0.9	98
70% Sodium fluosilicate, 30	21.8	49	18.0	52
Untreated	42.5		37.5	

Table 4.—Effectiveness of airplane sprays in controlling small salt-marsh caterpillars, Phoenix, Arizona, 1958.

Toxicant and Pounds per Acre (Emulsion Concentrates Used)	Larvae per Leaf 4 Days After Treatment	
	No.	Per Cent Control
Toxaphene, 4+DDT, 2	28.2	48
Dilan, 0.63	1.5	97
Parathion, 0.5	0.2	99
Untreated	54.0	—

Table 5.—Effectiveness of airplane sprays in controlling small salt-marsh caterpillars, Phoenix, Arizona, 1958.

Toxicant and Pounds per Acre (Emulsion Concentrates Used)	Larvae per Leaf After					
	2 Days		6 Days		13 Days	
	No.	Per Cent Control	No.	Per Cent Control	No.	Per Cent Control
Toxaphene, 6+DDT, 3	1.7	92	2.8	83	77.0	0
Dilan, 1	1.6	92	0	100	0.3	99
Parathion, 0.5	0	100	0	100	100.0	0
Untreated	20.6	—	16.6	—	39.0	—

Table 6.—Effectiveness of airplane sprays in controlling older salt-marsh caterpillars, Phoenix, Arizona, 1958.

Amount Toxicant and Pounds per Acre (Emulsion Concentrates Used)	Larvae per Terminal Foot of Plant	
	No.	Per Cent Control
Parathion, 0.75	0.2	90
Dilan, 0.9	1.0	52
Dylox, 2	0.0	100
Sevin, 2	0.1	95
Untreated	2.1	—

Table 7.—Population buildup of salt-marsh caterpillars in plots that received 12 weekly applications of insecticidal dusts. Phoenix, Arizona, 1958.

Dust Treatments and Per Cent Used (30 Lb./A.)	Larvae per Terminal Foot of Plant		
	No.	Per Cent Control	Per Cent Leaves Consumed
Toxaphene, 15+DDT, 5+sulfur, 40	0.4	50	40
Dieldrin, 2+DDT, 10+sulfur, 40	0.6	25	50
Sevin, 7.5+sulfur, 60	0.1	88	5
Malathion, 5+DDT, 10+sulfur, 50	0.8	0	40
Endrin, 1.5+Methyl, 1.5, Parathion	0.2	75	7
Untreated	0.8	—	80

malathion dust and a dust containing 2% dieldrin plus 10% DDT were ineffective (table 7).

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The Influence of Selection Pressure on the Development of Resistance in Populations of *Tetranychus telarius* (L.)[1]

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ABSTRACT

A program of building strains of the two-spotted spider mite, *Tetranychus telarius* (L.), resistant to parathion under 90° F. constant temperature has been carried through 45 generations treating all stages by the aerosol method and allowing the survivors to interbreed. All strains were initially sampled from a highly susceptible wild population (N-3) and were distributed among two levels of selective intensity (high and low). The results of this program lead to the following conclusions. (1) Resistance develops more rapidly under high selection pressure; (2) Under regular periodic selection the same level of resistance is reached in time, regardless of selection pressure; (3) Low selection pressure in this program appears ultimately to produce more homogeneity than high selection pressure. Greater homogeneity may be due to chance variability or a difference in the "quality" of resistance as defined in the text; (4) High levels of resistance can be demonstrated after one severe selection. Early appearance of resistance may be related to the failure of the Control Strain to demonstrate 100% mortality when exposed to selection treatments.

Current information on house flies (*Musca domestica* L.) and *Drosophila* suggests that the higher the selection pressure the greater the rate of development of resistance. In general, however, selection pressure does not appear to influence the final level of resistance (Tattersfield *et al.* 1953, Patton & Whittemore 1950).

The exception to this generality was reported by King (1954) who achieved a lower resistance level with a high selection pressure than with an intermediate or a low selection pressure. The lower resistance level resulting from a high selection pressure was considered by Crow (1957) to result from a small number of survivors and a consequent loss of genetic variability.

The data reported in this paper suggest that the influence of selection pressure on the development of resistance in the two-spotted spider mite, *Tetranychus telarius* (L.), is substantially the same as that which occurs in the house fly and *Drosophila*.

EXPERIMENTAL PROCEDURE.—A laboratory population of the two-spotted spider mite arbitrarily designated as N-3 was started from a wild population collected on hollyhock (*Althaea rosea* Cav.) at Slaterville Springs, New York in June 1954. Repeated tests on this laboratory population indicated a high degree of susceptibility to parathion.

By using a method of representative sampling of population N-3, five test populations were established. Each of the new populations was held separately in a 90° F. constant temperature cabinet.

The maintenance of these populations included elaborate safeguards against contamination during holding and during transfer to fresh bean plants. Each population was maintained on four flats of bean plants. A planned rotation of the flats was employed to assure equal opportunity for interchange of mites within each population. In order to affect transfer to fresh food all of the mite-infested leaves of the old plants were placed on new bean plants. Because of overlapping of the old mite-infested leaves it was necessary periodically to rearrange the leaves to give all mites an equal opportunity to move to the fresh foliage. When the mites were transferred, the remaining old bean stems and soil were discarded and the flats were scrubbed and rinsed in hot water.

Four resistant strains and one non-resistant strain were developed from these populations. In order to identify these new strains the following designations and descriptions were made:

The Control Strain was a representative sample from population N-3, held at 90° F. and never selected with chemicals. Concentration-mortality tests were completed on N-3 at the initiation of the program and on the Control Strain at the 27th and 45th generations.

Strain S-27 was a representative sample from population N-3 held at 90° F. and selected for resistance with a lower selection pressure than Strain S-88. The initial selection resulted in 27% mortality.

To determine the LC_{50} and LC_{95} and slope values of Strain S-27 concentration mortality tests were run at the 27th and 45th generations of selection. Selection was discontinued after the 45th generation.

Strain S-88 was a representative sample from population N-3, held at 90° F. and selected for resistance with a higher selection pressure than Strain S-27. The initial selection resulted in 88% mortality. As with Strain S-27 concentration mortality tests were run at the 27th and 45th generation of selection. Selection was discontinued after the 45th generation.

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Strain S-86 was a representative sample from population N-3, held at 90° F. and selected for resistance with an initial selection pressure similar to Strain S-88. Subsequent selections were much higher than Strains S-88 or S-27. The initial selection resulted in 86% mortality. Concentration-mortality tests were run at the 12th generation when selection was discontinued.

Strain S-95 was a representative sample from population N-3, held at 90° F. and selected for resistance with a very high selection pressure. The initial selection resulted in 95% mortality. Subsequent selection concentrations were higher than all other strains. Concentration mortality tests were run at the 12th generation when selection was discontinued.

Selection for resistance in these test populations of two-spotted mites was accomplished by treatment with parathion aerosol, in an aerosol chamber. Treatment of mite populations was scheduled to occur every third generation in order to allow the strain to recover after selection. Numerous tests had demonstrated that one generation occurred every 7 days under the holding conditions employed. The concentration of parathion was increased at almost every selection.

The aerosol bomb used in dispersing the parathion consisted of a 100-cc. graduated glass cylinder, an electrically operated solenoid valve and a No. 5 oil burner nozzle. The solenoid valve was actuated by an electrical timer. For short periods of emission, when the total contents of the cylinder were not exhausted, the mean for five consecutive emissions was 3 ml. per second with a standard error of ±0.319 ml. This rate did not vary with concentration up to 6%. Consequently, this rate was used to compute the number of milligrams of technical parathion dispersed for a given time at 0.02%, 1% and 2% technical parathion. The correlation between emission time and mortality in the N-3 population was then established as a guide for initial selection.

The mortalities that occurred at different locations in the aerosol chamber were computed after the completion of every aerosol treatment. From these data it was observed that although some variation did occur between locations no location was consistently high or low. To support these conclusions further an analysis of variance was completed on data collected from repeated tests conducted over several days. The analysis indicated that there were no significant differences in mortality (at the 1% level) between locations in the chamber.

The effect of each selection was determined by establishing on separate plants, prior to each selection, representative samples of mites from the strain to be selected, and from the Control Strain. The two samples and the strain to be selected (S-27, S-88, S-86 or S-95) were then all subjected to the same selection treatment. After treatment, the samples and the selected strain were held for 3 hours in the aerosol chamber. At the end of this time the selected strain was returned to the proper constant temperature cabinet and the samples were held for 24 hours at the same temperature as the selected strain. At the end of the 24-hour period the samples were counted to determine per cent mortality. The number of larvae was generally low limiting the evaluation to adults and nymphs. Usually over 1,000 adults plus nymphs were counted after each treatment. Eggs were not counted because of the relative ineffectiveness of parathion as an ovicide at the concentrations used (Ebeling & Pence 1954). The data obtained from the sample populations are presented in tables 1, 2 and 4.

Table 1.—Response of samples from strains S-88 and control to parathion aerosol applications. Cornell University, Ithaca, N. Y., 1956.

No. of Generations	Multiple of Base Concen.[a]	Strain Sampled	Total No. of Adults and Nymphs	Per Cent Mortality: Adults and Nymphs
6	2.0	S-88	1,606	88.0
		Control	791	92.8
12	1.7	S-88	2,681	50.8
		Control	1,102	69.9
15	1.7	S-88	1,630	29.3
		Control	1,379	50.5
18	3.0	S-88	1,231	44.8
		Control	1,231	96.2
21	4.0	S-88	1,249	67.7
		Control	483	88.6
24	4.7	S-88	1,442	17.5
		Control	1,182	91.8
27	5.3	S-88	1,292	9.7
		Control	1,246	97.7
30	249.2	S-88	1,323	48.5
		Control	322	99.4
33	846.1	S-88	1,224	79.0
		Control	1,170	99.9
36	997.0	S-88	1,216	56.5
		Control	1,106	99.5
39	1,250.4	S-88	795	90.9
		Control	2,141	99.1
45	4,985.5	S-88	715	59.6
		Control	385	98.7

[a] For purposes of comparison the base concentration was chosen as 0.228 mg. of technical parathion.

In addition to assaying the effectiveness and progress of each selection, concentration-mortality tests were completed on the strains at convenient intervals (the 12th or 27th and 45th generations) in order to establish a log-probit line. Each concentration-mortality test summarized in table 3 is a weighted average of four separate concentration-mortality tests run on 4 different days. Each test consists of a total of eight treatments (concentrations) with three replicates in each treatment or check. A replicate consisted of the treated mites found on the two primary leaves of a young bean plant. All tests were held before and after treatment in a cabinet held at a constant temperature of 90° F.±3°. Each plant was dipped in a suspension of parathion prepared by dissolving an appropriate amount of parathion in acetone. The initial acetone:water ratio was 1:9. Triton X-100 was held at a constant 1:10,000 at every dilution. No attempt was made to account for mites lost in the dipping or holding, since previous experimentation demonstrated

Table 2.—Response of samples from Strains S-27 and control to parathion aerosol applications. Cornell University, Ithaca, N. Y., 1956.

No. of Generations	Multiple of Base Concen.[a]	Strain Sampled	Total No. of Adults and Nymphs	Per Cent Mortality Adults and Nymphs
6	1.0	S-27	886	27.5
		Control	195	47.2
12	1.0	S-27	1,971	53.4
		Control	1,092	49.6
15	1.0	S-27	1,516	47.3
		Control	1,259	55.3
18	1.7	S-27	852	63.5
		Control	1,280	84.1
21	1.3	S-27	1,456	28.2
		Control	442	95.5
24	1.7	S-27	1,272	15.4
		Control	926	85.9
27	2.4	S-27	1,155	25.3
		Control	603	91.8
30	83.1	S-27	1,256	25.0
		Control	427	99.9
33	132.9	S-27	1,399	18.5
		Control	1,236	99.1
36	166.1	S-27	1,241	13.6
		Control	1,297	98.3
39	249.2	S-27	1,204	26.2
		Control	1,205	97.4
45	2,370.4	S-27	1,020	12.5
		Control	640	97.8

[a] For purposes of comparison 0.228 mg. of technical parathion was chosen as the base concentration.

no difference between parathion concentration-mortality curves which included or excluded these lost mites.

ANALYSIS OF DATA.—The mortality counts obtained from the samples taken at each selection were expressed as per cent mortality. No other statistical treatment of these data was performed.

Probit analysis (Finney 1952) was used to evaluate the concentration mortality data and provide the log-probit line. This method provided a more critical evaluation of change in response to parathion. Because of the increased variability and consequent unreliability of points below 20% and above 80% mortality, no test was considered adequate unless at least four points fell within these limits. All the log-probit lines reported in this paper consist of at least four pooled tests. On occasion, more than four tests were pooled if excessive scatter was encountered. Tests that demonstrated extreme scatter attributable to difficulties in the testing technique were discarded. All mortalities were corrected for check mortality by Abbott's formula.

The LC_{50} and slope values have been used as means of comparing the strains. The LC_{95} was calculated for each strain primarily because of its usefulness in reproducing the line. The 95% fiducial limits as included in these calculations define the zone of error and indicate the variability of the LC_{50}, LC_{95}, and slope values of the calculated line. In comparing one strain with another, values were considered significantly different if no overlapping occurred between the fiducial limits of the compared values.

DEVELOPMENT OF RESISTANCE IN STRAIN S-88.—A summary of the response to selection of Strain S-88 is presented in table 1 and figure 1. The response during selection was erratic reflecting the continual change of resistance level and selection concentration with succeeding generations. It is apparent that resistance developed rapidly during the course of selection for there was a 50% drop in mortality with an increase of 1.2 times the previous concentration from the 21st to the 24th generation. The same general pattern of sizable increase of concentration accompanied by a mortality decrease is apparent from the 33rd to the 36th and from the 39th to the 45th generation of selection.

The decrease in mortality which occurred from the 6th to the 15th generation is a reflection, in part, of a lowering of the selection concentration in an attempt to adjust the selection pressure to a more desirable level. It is probable that the response of Strain S-88 to this lowering of the concentration was more dramatic than that observed in the Control Strain because of the increased numbers of resistant individuals in Strain S-88. The Control Strain never exhibited 100% mortality to the selection concentrations used to select Strain S-88.

A summary of the concentration-mortality responses for Strain S-88 at the 27th and 45th generation is presented in table 3. On the basis of LC_{50} comparisons with

Table 3.—Summary of concentration-mortality data for Strains N-3, Control, S-27, S-88, S-86 and S-95 in response to parathion. Cornell University, Ithaca, N. Y., 1956.

Strain	Generation of Selection	LC_{50}	95% Fiducial Limits	LC_{95}	95% Fiducial Limits	Slope	95% Fiducial Limits
N-3	0	0.00041	0.00045–.00037	0.0025	0.0034–.0019	2.063	2.384–1.743
Control	27	.00049	.00052–.00046	.0045	.0055–.0037	1.703	1.837–1.569
Control	45	.00026	.00033–.00020	.0015	.064 –.0021	.998	1.198– .798
S-27	27	.0142	.0151 –.0134	.0849	.1039–.0694	2.116	2.339–1.892
S-27	45	.0235	.0251 –.0220	.0562	.0665–.0475	4.345	5.104–3.585
S-88	27	.0186	.0198 –.0176	.0856	.0989–.0741	2.483	2.724–2.242
S-88	45	.0234	.0250 –.0221	.0874	.0160–.0721	2.882	3.293–2.472
S-86	12	.0193	.0208 –.0181	.0735	.0934–.0578	2.840	3.260–2.420
S-95[a]	12	.0232	.0270 –.0201	.0771	.1152–.0578	3.164	4.034–2.293

[a] Heterogenous data—all variances multiplied by heterogeneity factor (Finney 1952).

the Control Strain, Strain S-88 was 38-fold resistant at the 27th generation and 91-fold resistant at the 45th generation. The slope values of the Control Strain and Strain S-88 suggest that the Control Strain became more heterogeneous as the program progressed while Strain S-88 tended to be homogeneous.

DEVELOPMENT OF RESISTANCE IN STRAIN S-27.—The manner in which this strain was selected was similar to that of Strain S-88. The only intended difference was that of the selection pressure used in selection. A summary of the response of Strain S-27 to selection is presented in table 2 and figure 2. The response of Strain S-27 to selection was also erratic, but not as pronounced as in Strain S-88, probably because of the lower concentrations used throughout selection. Increases in concentration accompanied by mortality decreases are evident in generations 21 to 24, 30 to 33 to 36 and 39 to 45. That resistance developed more slowly in Strain S-27 than in Strain S-88 is evident as early as the 15th generation, but is especially marked in the 24th and 27th generations. At the 24th generation Strain S-27 was selected with a concentration approximately one-third that used to select Strain S-88 at the 24th generation yet the mortality responses were about the same. The difference in resistance level is further emphasized in the 27th generation where Strain S-27 is selected with a concentration approximately one-half that used to select Strain S-88, yet the mortality response of Strain S-27 is notably higher than that of Strain S-88.

A summary of the concentration-mortality data for the Control Strain and Strain S-27, completed at the 27th and 45th generations, is presented in table 3. If the LC_{50} values are compared Strain S-27 appears 29 and 91 times more resistant than the Control Strain at the 27th and 45th generations, respectively.

The slope of the log-probit line of Strain S-27 is noticeably steeper than Strain S-88 at the 45th generation suggesting that a more homogeneous population resulted from the selection of Strain S-27. The reason for the steeper slope in Strain S-27 is not obvious, but may be a

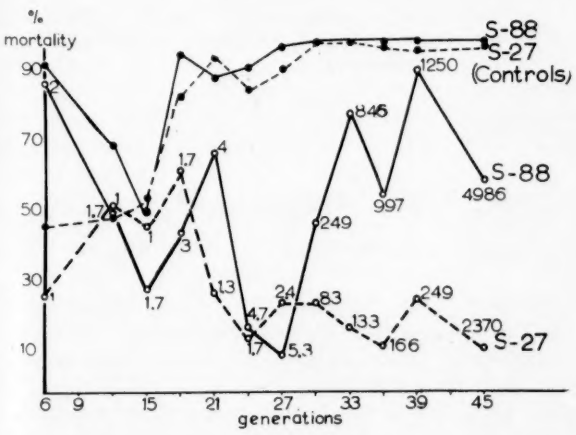

FIG. 1.—The response of samples from *Strains S-27, S-88,* and *Control* to parathion aerosol applications throughout selection with parathion aerosols. The figure accompanying each response represents a multiple of the base selection concentration (0.228 mg. of techical parathion).

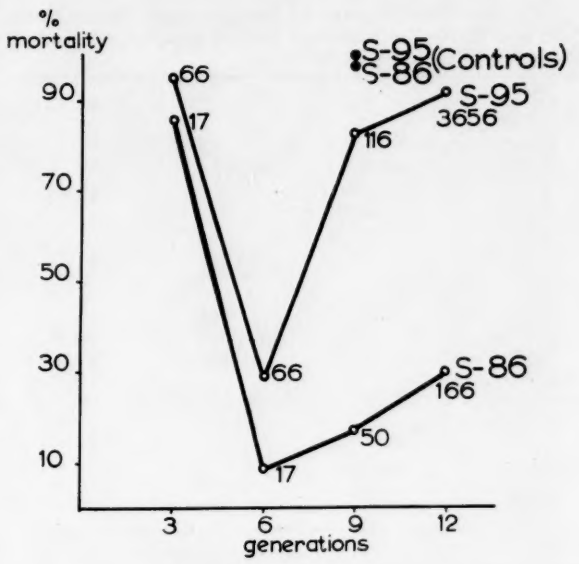

FIG. 2.—The response of samples from *Strains S-86, S-95* and *Control* to parathion aerosol applications throughout selection with parathion aerosols. The figure accompanying each response represents a multiple of the base selection concentration (0.228 mg. of technical parathion).

subtle demonstration of a difference in the "quality" of resistance resulting from selection pressure or it may be a chance variation. By "quality" of resistance we refer to population characteristics other than resistance level and rate of resistance development. Quality of resistance would then include responses such as cross resistance patterns, the persistence of resistance, and differential response of the strain under different environmental conditions, as well as other responses which measure biological characteristics.

The length of the life cycle of either the resistant strains or the Control Strain throughout the course of the study did not change from the original N-3 population.

DEVELOPMENT OF RESISTANCE IN STRAINS S-86 AND S-95.—The suggestion of measurable resistance levels in the early generations of selection of Strain S-88 prompted the selection of these two strains at comparable and higher levels of selection than that used to select Strain S-88. The responses of these strains to selection are presented in table 4 and figure 2.

The development of resistance in these strains after only one selection is evident both from the response of each strain to the high concentration used in subsequent selections and the concentration-mortality information obtained at the 12th generation. A summary of this concentration-mortality data is presented in table 3. Both these strains demonstrated resistance levels at the 12th generation comparable to Strains S-27 and S-88 at the 45th generation. Subsequent selections of other strains with parathion suggest that the LC_{50} values here reported are plateau levels for parathion selection.

It is notable that severe selection of Strain S-95 did not impair the degree of resistance developed in it, which appears contradictory to what would be expected from a consideration of King's (1954) experiences. However,

Table 4.—The response of samples from Strains S-86, S-95 and Control to parathion aerosol applications. Cornell University, Ithaca, N. Y., 1956.

No. of Generations	Multiple of Base Concen.[a]	Total No. of Adults and Nymphs	Per Cent Mortality Adults and Nymphs
Sample of Strain S-86			
3	16.6	1,190	85.8
6	16.6	609	9.4
9	49.9	811	17.1
		859[b]	98.9[b]
12	166.0	429	29.8
Sample of Strain S-95			
3	66.4	1,205	95.2
6	66.4	573	29.3
9	116.36	561	82.9
		1,164[c]	99.2[c]
12	361.1	397	91.7

[a] For purposes of comparison 0.228 mg. of parathion was chosen as the base concentration.
[b] Control Strain S-86.
[c] Control Strain S-95.

there were large numbers of survivors following even severe selections.

At the termination of selection the slopes of all the selected strains were steeper than the N-3 Strain and the Control Strain. In addition the LC_{95}'s of the selected strains are significantly different from the LC_{95}'s of the Control Strain and the N-3 Strain so that the development of resistance in these strains does not demonstrate what Hoskins & Gordon (1956) consider a characteristic increase in heterogeneity during selection. However, the meaning of the slope value in the characterization of mite populations is still subject to clarification. Consequently, the slope must be interpreted with some reserve. This is particularly true when comparing the response of mite populations with the response of other organisms selected with different techniques and chemicals as well as characterized with a log-probit curve obtained by the use of different experimental techniques. If the slope values obtained on these mite populations can be validly compared with those obtained on *Drosophila* then the lack of increased heterogeneity during the selection of these strains may suggest a difference in the pattern of resistance development in mites as opposed to *Drosophila*. On the other hand, this lack of heterogeneity may also be caused by our failure to establish a log-probit relationship at the period of greatest heterogeneity.

Response of the Control Strain.—The summary of concentration mortality data for the N-3 population and the Control Strain is presented in table 3. These data demonstrate a slight trend toward increased susceptibility and an increase in heterogeneity in the Control Strain from the 27th to the 45th generation which is evident from the increase in LC_{50} and the decrease in slope value. The close agreement of the LC_{50}, LC_{95} and slope values of the N-3 population and the 27th generation of the Control Strain suggests that the sampling procedure used to establish the Control Strain from the N-3 populations was unbiased. All the other strains established from the N-3 population were established with a similar sampling procedure.

The failure of the Control Strain to exhibit 100% mortality during selection of any strain may be the result of resistant individuals in the Control Strain and/or the inherent distribution problems of the aerosol method, such as poor deposit on the undersurface of the leaves and on vertical leaf surfaces.

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Response of Different Strains of the Green Peach Aphid to Malathion[1]

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Abstract

Several strains of the green peach aphid (*Myzus persicae* (Sulz.)), reproducing only parthenogenetically, showed some differences in the amounts of malathion required to kill them. Aphids from Nebraska, Illinois, and Maryland were initially much less tolerant to malathion than aphids from California or Washington. Aphids bred from those surviving treatment showed some increase in tolerance over the original strains, but continued selection did not give further increases. Aphids with wing pads withstood malathion applications better than aphids without wing pads.

In the Pacific Northwest the green peach aphid (*Myzus persicae* (Sulz.)) has been difficult to control (Landis *et al.* 1958) and spreads the destructive leaf roll disease of Russet Burbank potatoes even when populations are very low. Stephenson (1954) found that aphids collected from parathion-treated potato fields in the

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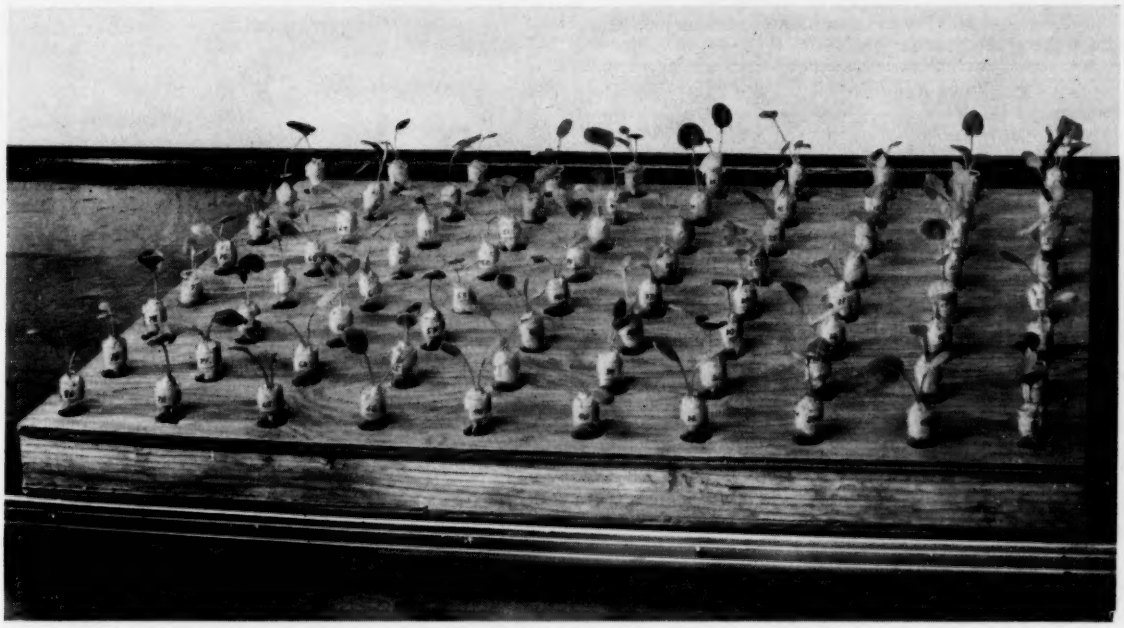

FIG. 1.—Sprayed broccoli plants awaiting aphid counts.

Yakima Valley and brought to the laboratory at Pullman, Washington, were consistently harder to kill with parathion than aphids collected locally. Anthon (1955) reported that this aphid had apparently developed a resistance to organic-phosphorus insecticides on peach trees in north-central Washington.

In March 1957 studies were begun at Union Gap, Washington, on the response to different green peach aphid strains to malathion. The objectives were to determine (1) whether aphids from different localities differed appreciably in their susceptibility to malathion, (2) whether successive generations, on being repeatedly sprayed, would develop increasing resistance, and (3) whether aphids with wing pads were harder to kill than those without wing pads.

METHODS.—Various strains of the green peach aphid were maintained and bred on caged broccoli plants in the greenhouse. Each cage was 8×8×16 inches, covered on the top and three sides with Saran screen cloth. A vertically sliding glass panel closed the front. Each cage was set on a 1-gallon can of soil in which a broccoli plant was growing. The can stood in a saucer, enabling the plant to be subirrigated.

Seedling broccoli plants, grown in sand, were placed in shell vials $\frac{3}{4}$ or 1 inch in diameter (fig. 1). Each plant was first wrapped with cellulose tissue to hold it erect and to form a plug to the vial. The vials contained tap water during most of the tests, although dilute Hyponex, a balanced plant nutrient, was used in the first tests. The aphids were transferred with a camel's-hair brush to the cellulose at the base of each plant, and established themselves on the leaves. The plants were infested one day and sprayed the next. There were 8 or 10 plants for each dosage, with a pre-treatment average of about 17 aphids per plant, or 600 per strain. Usually three dosages were tested, but sometimes there were four. The plants were sprayed in pairs as they revolved on a turntable (fig. 2), one plant of each line or strain being compared.

Commercial emulsifiable concentrates containing 50% or 57% of malathion were used. A 1% emulsion was prepared at the beginning of each test and diluted to give the dosage desired. The sprays were applied in descending order of concentration, the spray gun being washed with acetone and rinsed with water after each treatment.

The sprays were applied at 5 pounds pressure through a DeVilbiss spray gun (type T.G.A. Series 502) having a No. 90 nozzle. After treatment the plants were left in the laboratory for 24 hours before mortality records were made.

Mortality results were judged by the per cent reduction of treated aphids and also by LD-75 values obtained by plotting the mortality data on logarithmic-arithmetic probability paper and fitting a dosage-mortality line freehand. The LD-75 values were read directly from this line.

APHIDS TESTED.—The strains of greenhouse-bred aphids were obtained as follows: Washington aphids were brought in from the laboratory garden at Yakima in September 1956. Nebraska aphids were collected by the author in Omaha in October 1956. California aphids were taken from a head of romaine originating in central California and obtained in a Yakima grocery store in March 1957. Illinois aphids were received from Georgetown on October 24, 1957, North Carolina aphids from Oxford on January 6, 1958, and Maryland aphids from Beltsville on March 20, 1958. In one line of each strain the aphids in the breeding stock were never treated with insecticide. Attempts were made from time to time to establish additional lines from progeny of the treated aphids.

RESPONSE OF APHIDS FROM DIFFERENT LOCALITIES.—In the 1957 tests the California aphids seemed more tolerant to malathion than those from Washington or Nebraska (table 1).

Table 1.—Susceptibility to malathion of various untreated strains of the green peach aphid, 1957.

Date and Dosage (p.p.m.)	Per Cent Reduction		LD-75 (p.p.m.)	
	California	*Washington*	*California*	*Washington*
March 21				
1,000	98	100	285	140
500	93	98		
250	59	95		
Check	83[a]	39		
March 29				
500	93	98	105	70
250	85	97		
125	88	98		
62	29	64		
Check	52[a]	29[a]		
		Nebraska		*Nebraska*
April 12				
125	71	99	>125	15
62	24	100		
15	32	75		
Check	9[a]	10[a]		
April 24				
125	75	100	125	<15
62	69	99		
15	27[a]	89		
Check	14[a]	25[a]		

[a] Increase.

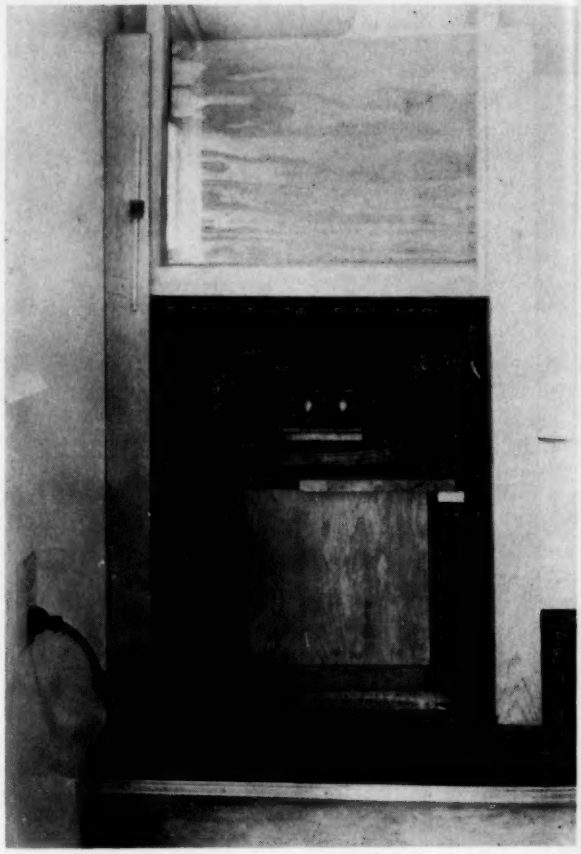

FIG. 2.—Spray hood and turntable used for testing tolerance of green peach aphid to malathion.

In 1958 the Washington strain, continued from 1957, also seemed to have a greater tolerance than the aphids from Illinois or North Carolina (table 2). However, wide variations in LD-75 values within strains do not permit conclusions concerning any real difference between strains. As shown in figure 3, the small differences between strains reared continuously in the greenhouse tended to disappear in the summer of 1958.

TESTS OF PROGENY FROM TREATED APHIDS.—The Nebraska aphids were highly susceptible to malathion, especially in the early tests. Treating parent aphids of this strain with malathion caused a significant increase in tolerance of the progeny as indicated by mortality records (table 3). Tolerance tended to increase slightly with the treatment of successive generations. However,

Table 2.—Susceptibility to malathion of strains of the green peach aphid not previously treated, 1958.

Dosages (p.p.m.)	Date Applied	Number of Aphids		LD-75 (p.p.m.)	
		Washington	*Illinois*	*Washington*	*Illinois*
62, 31, 15	Jan. 9	533	485	54	44
	Jan. 13	659	706	50	—
	Jan. 16	435	814	48	—
62, 15, 7	Jan. 15	402	693	34	—
62, 15, 4	Jan. 21	509	549	44	23
	Jan. 23	443	536	66	50
125, 62, 15	Jan. 27	648	408	70	55
	Feb. 11	448	618	75	25
		Washington	*North Carolina*	*Washington*	*North Carolina*
125, 62, 15	Jan. 29	523	645	95	27
	Feb. 13	283	460	21	—
	Mar. 6	679	711	85	30
	Mar. 11	805	880	90	90
	Mar. 13	653	714	77	47
		Illinois	*North Carolina*	*Illinois*	*North Carolina*
62, 15, 7	Feb. 18	410	616	19	—
62, 31, 15	Feb. 26	149	143	21	45
125, 62, 15	Feb. 20	582	629	41	38
	Feb. 27	679	766	53	24
	Mar. 4	569	738	—	43

Table 3.—Effect of malathion sprays at 15 parts per million on previously treated and untreated lines of Nebraska aphids, 1957.

Date of Application	Number of Previously Treated Generations	Per Cent Reduction	
		Treated	Untreated
May 7	1	65	79
15	1	85	95
22	1	100	100
June 6	2	75	99
11	1	95	98
	1	99	98
14	2	79	96
Sept. 26	4	11	48
Oct. 15	4	15	40
Average	—	69.3[a]	83.7[a]

[a] L.S.D. at 5% level 9.3.

the LD-75 values (fig. 3, C) show little difference between the treated and untreated lines. Lines previously treated with malathion had a higher tolerance than untreated lines of the same strains in 14 out of 18 tests and in 5 of 6 strains (table 4). However, as many as seven previously treated generations of California aphids did not show any increase in tolerance.

Aphids from untreated field plots were more susceptible

Table 4.—Malathion tolerance of progeny of various strains of the green peach aphid previously treated with malathion compared with that of aphids from untreated lines.

Date of Application	Number of Previously Treated Generations	LD-75 (p.p.m.)		Tolerance Ratio: Treated to Untreated
		Previously Treated	Untreated	
1957		*Washington*		
May 2	1	120	73	1.6
16	1	70	46	
1957		*Nebraska*		
June 14	2	12	6	1.2
	2	12	4	
Aug. 22	4	73	73	
Sept. 26	4	40	27	
1957		*California*		
July 26	3	61	45	0.9
Sept. 10	4	31	53	
11	5	33	55	
Oct. 11	6	40	46	
Nov. 18	7	61	40	
1958		*Illinois*		
Feb. 26	1	45	21	1.7
Mar. 18	1	100	42	
27	1	65	61	
June 20	3	275	155	
1958		*North Carolina*		
May 6	2	94	80	1.2
1958		*Maryland*		
Apr. 30	1	40	10	8.6
May 20	2	105	7	

Table 5.—Malathion tolerance of aphids from fields previously treated with parathion or demeton and from untreated fields, Washington, 1957.

Date of Malathion Application	LD-75 (p.p.m.)	
	Treated	Untreated
	Parathion	
June 28	85	60
July 3	79	55
	Demeton	
5	83	73

to malathion than aphids from plots treated with parathion or demeton (table 5).

APHIDS WITH WING PADS.—Field experiments indicated that aphids with wing pads were more difficult to control with insecticides than those without wing pads. To investigate this point, aphids with and without wing pads were removed from potatoes growing in the laboratory garden and placed on small broccoli plants for testing. Large numbers of aphids with visible wing pads could only be found during 1 week, from July 26 to August 2. This period was too short to enable many tests to be made. However, in each of the four tests made, the aphids with wing pads showed a higher tolerance for malathion (table 6).

DISCUSSION.—All aphid reproduction in these studies was asexual. Therefore, the genetic basis for variation, insofar as the recombination of genetic factors in successive sexual generations is involved, was lacking. However, it is interesting that Stern & Reynolds (1958) in discussing the resistance that the spotted alfalfa aphid (*Therioaphis maculata* (Buckton)) has seemingly ac-

Table 6.—Susceptibility to malathion of aphids with and without wing pads, 1957.

Date and Dosage (p.p.m.)	Per Cent Reduction		LD-75 (p.p.m.)	
	With Wing Pads	Without Wing Pads	With Wing Pads	Without Wing Pads
July 26				
125	67	92	155	62
62	37	73		
31	35	53		
Check	8	8		
July 30				
125	89	85	63	27
62	67	79		
31	51	77		
Check	30	25		
July 31				
125	73	94	75	45
62	84	75		
31	54	72		
Check	22	23		
Aug. 2				
125	89	96	60	40
62	79	92		
31	56	69		
Check	44	30		
Average	—	—	88.3	43.5

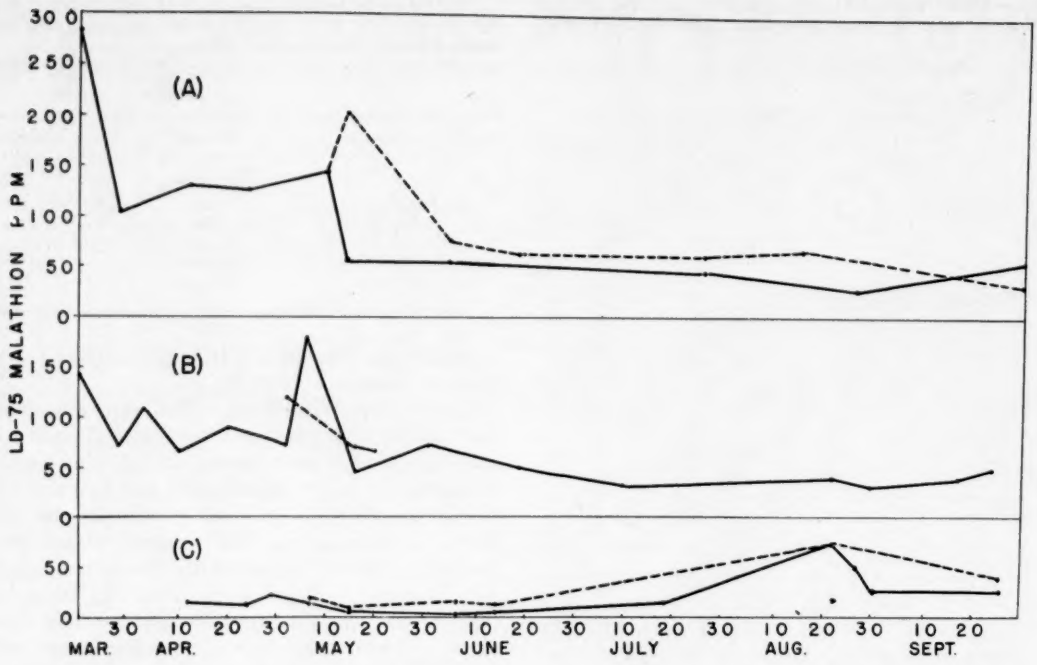

FIG. 3.—Dosage-mortality responses of different strains of the green peach aphid in laboratory tests: (A) California, (B) Washington, (C) Nebraska. Solid lines progeny of untreated aphids; broken lines progeny of aphids treated with malathion.

quired for organic-phosphorus insecticides, suggested that even under clonal development a resistant strain might have arisen through mutation.

Hoskins & Gordon (1956) suggested that true resistance to an organic-phosphorus insecticide should involve at least a twofold increase in lethal dosage. Except possibly for the Maryland strain, the increases observed in these tests were not that great (table 4). Hoskins and Gordon introduced a new term, "vigor tolerance," for the increase in vigor an insect may acquire through the action of selective factors such as extremes of temperature, dryness, poor food, or a chemical. This vigor tolerance is much less than their concept of true resistance. The initial application of malathion in these tests may have acted as the selective factor, leaving those aphids possessing vigor tolerance as survivors. Hoskins and Gordon's statement that "field observations that once successful practices are no longer effective are ample evidence [of resistance] to the practical entomologist" supports the opinion of Anthon (1955) that for practical purposes the green peach aphid in central Washington has acquired resistance to organic-phosphorus insecticides.

In recent years it has been necessary to use twice as much parathion per acre as was used before for commercial control of this aphid on potatoes in central Washington. This insect has consistently shown a tendency for increased tolerance to organic-phosphorus insecticides in both the laboratory and the field. This increase, though neither large nor cumulative, is nevertheless important from an economic standpoint. Aphids that survive applications early in the season, when potato vines are small, may also survive later applications because of vigor tolerance and the protection afforded by increased plant growth. They may produce populations sufficient to spread the potato leaf-roll disease.

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Biology and Evaluation of Certain Predators of *Therioaphis maculata* (Buckton)[1]

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ABSTRACT

Eleven species representing five families of common native predators of the spotted alfalfa aphid, *Therioaphis maculata* (Buck.), were investigated to determine their beneficial effects in the Manhattan, Kansas, area. Life histories, daily feeding rates on different sizes of aphids and field populations were determined for most immature and adult predators. A comparison was made of warm and cold temperature effects on predator feeding. A relative value of the predators in the field was determined by multiplying the average daily consumption of aphids times the field populations. A lady beetle, *Hippodamia convergens* G.-M., was the most important of all insects studied. Chrysopidae (green lacewings), Anthocoridae (minute pirate bugs), Nabidae (damsel bugs) and Syrphidae (flower flies), in that order, were next most efficient following coccinellids. Low field populations were the primary reason for lower efficiency of the latter groups.

Life histories of several common predators of spotted alfalfa aphids, *Therioaphis maculata* (Buck.), are known but records of other species are insufficient. Balduf (1935, 1939) gave a comprehensive report on the bionomics of several species of coccinellids and of chrysopids. Jones (1922) in Colorado reported briefly on life history and feeding habits of several syrphids. In Kentucky, Garman & Jewett (1914) recorded detailed life histories of *Nabis ferus* (Linn.) and they, and Marshall (1930) in Kansas, gave accounts of the life history and habits of *Orius insidiosus* (Say).

When the present study was initiated, no information was available concerning actual feeding capacities of various predators on this aphid. Feeding predators large numbers of aphids each day indicated their potential capacities for this pest. Goodarzy & Davis (1958) in Utah and Nielson & Henderson (1959) in Arizona reported on the relative effectiveness of certain predators feeding on the aphid. Only mature aphids were used for their feeding studies.

MATERIALS AND METHODS.—The present study was conducted in a screened insectary located about one block from an official U. S. Weather Bureau Station and weather data recorded by this Station were used. Gravid females of the desired predator species were collected in the field to start rearings with the egg stage. When oviposition or hatching occurred, eggs or immature stages were individually caged.

Lady beetles and green lacewings were reared in modified pint ice cream cartons. The carton lid, with a $\frac{1}{4}$-inch hole in the center, was tacked to the upper crosspiece of the supporting apparatus as shown in figure 1. The lower removable crosspiece, held in position by "L hooks," supported test tubes containing tap water for a single vigorous alfalfa stem stripped of all but the terminal pair of trifoliate leaves. Cellucotton placed in the space between the stem and hole of the carton lid prevented escape of aphids or predators. A 2-inch hole in the inverted carton bottom was enclosed with 100-mesh nylon. Light from a fluorescent lamp suspended 18 inches above the apparatus and other rearing cages was supplied 8 hours each day. This method of rearing predators was thought to provide a more natural environment than using vials stoppered with cotton plugs.

Nabids (damsel bugs) deposit eggs within plant stems. In the above method, stems would shrivel and dry, trapping eggs before they could hatch. Substitution of a potted plant ensured that plant tissue surrounding the eggs would remain soft and allow them to hatch. Both nabid nymphs and larvae of Syrphidae (flower flies) were reared in $2\frac{3}{4}'' \times \frac{3}{4}'' \times 1''$ plastic boxes containing a trifoliate leaf.

Syrphid eggs were obtained by placing gravid females in a lantern globe screened at the top and placed over potted, blooming alfalfa plants infested with aphids. A small portion of a plant containing an egg was removed and placed in a plastic box. After eggs hatched, a blotter covering the bottom of each cage was moistened slightly each day. At times, added moisture appeared to give new incentive to larvae to feed.

A cage for rearing Anthocoridae (minute pirate bugs) was constructed from two vials, one of which had the bottom removed, and two cork stoppers (fig. 2). Both stoppers contained $\frac{1}{4}$-inch holes. The upper stopper was covered with nylon and glued, allowing air to enter the cage. Gravid females were placed in a "vial cage" to oviposit within the plant tissue. The females were later removed and the eggs allowed to hatch. At feeding time the cylinder along with both stoppers was removed from the vial and examined under a binocular microscope to check for molting and to count aphids not consumed or destroyed.

Predators, in general, feed on all instars of aphids. Consequently, the following sizes of aphids were selected: (1) small, representing first instar; (2) medium, representing third instar; (3) fourth instar (alate form); and (4) large, representing adults. Predators, upon hatching, were given a predetermined size and number of aphids to feed on. The number of aphids introduced into cages at approximately the same time each day was always more than the predators could consume. Aphids partially eaten, or killed but not eaten, were considered "consumed."

To evaluate predator effectiveness in the field, it was

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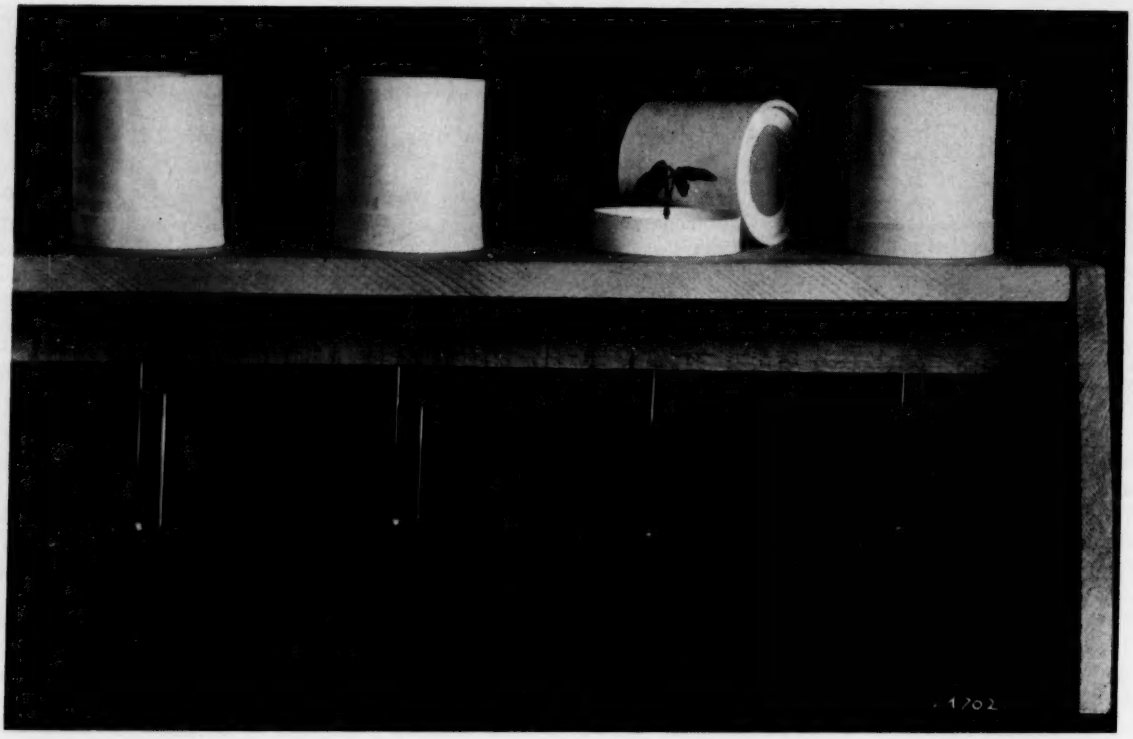

FIG. 1.—Apparatus supporting cages for rearing coccinellids and chrysopids and for nabid oviposition within alfalfa stems.

necessary to know the abundance of field populations in relation to numbers of aphids consumed during feeding experiments. Field surveys were made periodically during the 1957 and 1958 growing seasons. Because of excessive rainfall during 1957, only 1958 survey data were used. When fields were mowed within a few days before a survey, a comparable alternate field, previously located nearby, was used. A 15-inch net was used in 180° sweeps. Seventy-five sweeps were made covering a representative portion of each field. Fields averaged about 6 acres. A count of all predators as well as spotted alfalfa aphids was made after each 25 sweeps.

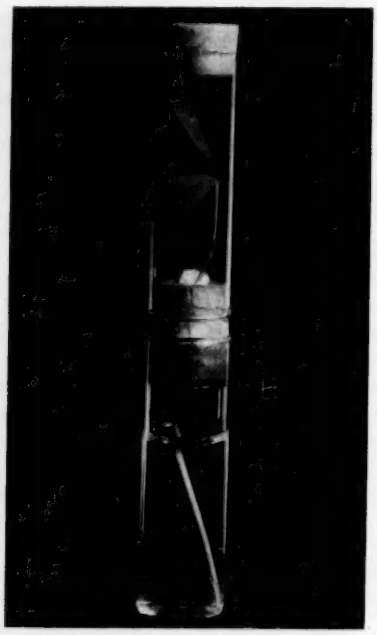

FIG. 2.—Vial cage (1 dram) for rearing and observing anthocorids.

A theoretical effectiveness of predators was calculated by multiplying the seasonal average number of predators per 75 sweeps obtained from the surveys times the average daily consumption of each predator (table 9).

RESULTS AND DISCUSSION.—*Coccinellidae.*—Table 1 shows the average length of the stages in life histories of six species of coccinellids starting with egg deposition and continuing until emergence of the adult beetle.

Average life history stages of 20 individuals of the most important species, *Hippodamia convergens* G.-M. were as follows: egg, 3 days; first instar, 2.1; second, 1.8; third, 1.7; fourth, 3.6; pupa, 4.8; and the period from egg deposition to adult emergence, 16.9 days. Similar data are given in the table for five other species of lady beetles. Temperatures averaged 79° F. during the period of life history studies.

Average daily consumption of various sizes of spotted alfalfa aphids by coccinellid larvae is shown in table 2. Feeding records were maintained throughout a large portion of the life span of each predator and covered the period of July to mid-November, 1957, and July to mid-September, 1958. Larvae of *H. convergens* consumed the highest number of all sizes of aphids and *Coleomegilla*

Table 1.—Average number of days for life history stages of six species of Coccinellidae. Manhattan, Kansas, 1957–58.

Species	No. of Cases	Average Life History (Days)							
			Larval instars						
		Egg	1	2	3	4	Total Larva	Pupa	Total Egg to Adult
Coleomegilla m. lengi	20	2.8 (2–3)[a]	3.0 (2–5)	2.0 (1–3)	2.3 (1–4)	3.5 (3–4)	10.7 (9–14)	3.9 (3–5)	17.4 (15–19)
Cycloneda munda	13	4.0 (4)	1.7 (1–3)	1.4 (1–3)	2.2 (2–3)	3.2 (2–5)	8.5 (7–12)	5.6 (5–7)	18.2 (16–22)
Hippodamia convergens	20	3.0 (3)	2.1 (1–3)	1.8 (1–2)	1.7 (1–2)	3.6 (3–4)	9.0 (8–10)	4.8 (3–6)	16.9 (14–18)
H. g. glacialis	7	2.6 (2–3)	2.4 (1–4)	2.1 (1–3)	2.1 (1–3)	4.6 (4–6)	11.3 (9–13)	4.6 (4–6)	18.4 (16–22)
H. parenthesis	18	2.3 (2–4)	2.0 (1–3)	1.4 (1–3)	2.4 (2–3)	2.9 (2–4)	8.7 (7–11)	4.0 (3–5)	15.0 (13–17)
Olla abdominalis	18	2.3 (2–3)	1.4 (1–3)	1.1 (1–2)	1.5 (1–2)	3.9 (3–6)	8.0 (6–10)	4.8 (4–6)	15.0 (12–19)

[a] Figures in parentheses give the range.

maculata lengi Timberlake, the lowest number. Small aphids were not fed to the larvae of *Hippodamia glacialis glacialis* (Fab.).

Average daily consumption of aphids by adults in warm (75° F.) and cold (45° F.) weather is given in table 3. In warm weather *H. g. glacialis* consumed the largest average number of aphids (122.1) per day while *H. convergens* consumed an average of 89.4 aphids per day, ranking second highest. The lowest number (30.4) was consumed by *Cycloneda munda* (Say). In cold weather *H. convergens* ate (1.0), the largest daily average number and *C. m. lengi* (0.5) the lowest number. *C. m. lengi* normally feeds to some degree on certain parts of a variety of plants, as well as many insects. Decreased feeding and other activity during cold weather is well known. Cold temperatures influenced all species, resulting in an average consumption of less than one aphid per day at an average temperature of 45° F.

Each of 9 males and 6 females of *H. convergens* ate an average of 2,912 and 5,665 large aphids, respectively, in 48 days. During that time each of two other females ate 7,688 and 7,107 large aphids. A female of *C. m. lengi* consumed 4,485 large aphids during a 120-day feeding period.

Nearly all oviposition continued until predator feeding was stopped November 8, 1957. During the period of oviposition temperatures often were low. Consequently, the data included many days on which no eggs were laid and thus lowered the daily avergae tremendously. Oviposition periods ranged from 1 day for *H. g. glacialis* to 63 days for *C. m. lengi*. The maximum number of eggs laid by a single female was: *C. m. lengi* 146; *C. munda* 46; *H. convergens* 871; *H. g. glacialis* 95; *Hippodamia parenthesis* (Say) 195; and *Olla abdominalis* (Say) 168. Studies of *C. munda* and *H. g. glacialis* were started later in the season and had less time for favorable oviposition before cold weather. The highest oviposition record was 871 eggs for *H. convergens*.

The average number of the six species of coccinellids collected per survey date is shown in table 4. *H. convergens* was the most abundant with a seasonal average of 14.6 per survey. It was never as abundant as during 1957, probably because of the lower population of aphids throughout the season. *C. m. lengi* was second highest in

Table 2.—Average daily consumption of various sizes of spotted alfalfa aphids by larvae of six species of coccinellids. Manhattan, Kansas, 1957–58

Species	Sizes of Aphids Fed					
	Large[a]		Medium[b]		Small[c]	
	No. of Cases	Average Daily Consumption	No. of Cases	Average Daily Consumption	No. of Cases	Average Daily Consumption
Coleomegilla m. lengi	9	24.1	7	15.6	1	31.7
Cycloneda munda	8	40.3	4	51.5	1	81.3
Hippodamia convergens	9	45.0	3	84.3	2	106.8
H. g. glacialis	3	41.3	3	43.6	—	—
H. parenthesis	7	34.5	5	38.5	6	58.2
Olla abdominalis	13	41.8	3	49.3	2	98.0

[a] Large size represents the combined averages of large and fourth-instar (alate form) aphids.
[b] Medium size represents third instar.
[c] Small size represents first instar.

Table 3.—Average daily consumption of large spotted alfalfa aphids by six species of adult Coccinellidae during the first 14 days after emergence in warm weather and during the last 14 days of feeding in cold weather. Manhattan, Kansas, 1957–58.

Species	Warm Period-Average 75° F.		Cold Period-Average 45° F.	
	No. of Cases	Daily Average Number Large Aphids Consumed	No. of Cases	Daily Average Number Large Aphids Consumed
Coleomegilla m. lengi	9	46.2	8	.5
Cycloneda munda	9	30.4	8	.8
Hippodamia convergens	15	89.4	3	1.0
H. g. glacialis	4	122.1	6	.6
H. parenthesis	7	53.9	13	.6
Olla abdominalis	12	45.0	16	.9

Table 4.—Average number of predators and spotted alfalfa aphids collected per 75 sweeps from six fields on dates indicated. Manhattan, Kansas, 1958.

Insects	Average Number of Specimens per 75 Sweeps on Dates Shown								Average
	6/27	7/8	7/26	8/11	8/19	8/28	9/18	10/2	
Coccinellidae[a]	39.3	9.0	27.6	45.0	29.6	17.4	14.2	9.4	24.0
Coleomegilla m. lengi	2.9	.7	.6	2.3	4.8	3.6	.8	.6	2.0
Cycloneda munda	.6	0	.4	0	0	0	.7	.6	.3
Hippodamia convergens	33.3	8.0	16.6	15.0	16.0	13.2	10.7	3.8	14.6
H. g. glacialis	0	0	0	.5	0	0	.2	0	.1
H. parenthesis	1.3	0	1.0	.2	1.3	.4	0	1.2	.7
Olla abdominalis	0	0	0	.5	.2	0	0	0	.1
Chrysopidae	1.1	2.5	8.6	31.8	12.6	4.2	27.5	15.4	13.0
Syrphidae	0	0	.6	.3	.6	.8	1.0	.2	.4
Nabidae	10.5	1.3	6.6	8.5	6.6	10.2	6.3	16.4	8.3
Anthocoridae	15.9	26.3	33.4	42.3	40.1	51.6	47.5	41.6	37.3
Spotted alfalfa aphids	.1	.3	4.4	149.3	140.0	39.6	82.3	57.0	59.1

[a] Includes both unidentified and identified coccinellids.

population with an average of only 2.0. *H. g. glacialis* and *O. abdominalis* were the least abundant with an average of 0.1 per survey. In 1958, coccinellids ranked first and anthocorids second in average population.

Chrysopidae.—There are four larval instars in the development of these two species, the last instar occurring within the cocoon. In this study the "cocoon" stage, which included the fourth instar, started when the larva spun the pupal case and ended with the emergence of the adult.

Average life histories obtained from 13 cases of *Chrysopa oculata* (Say) and 17 cases of *C. plorabunda* Fitch are shown in table 5. The average period from egg deposition to adult for the two species was 26 and 22.4 days, respectively. Studies were conducted during June, July and August, 1958, when temperatures averaged 77° F.

C. oculata and *C. plorabunda*, with eight records each, destroyed an average of 32.8 and 34.4 large aphids per day, respectively, during their larval periods (table 6), indicating that the larvae are of equal value as predators. Total large aphids consumed by *C. oculata* larvae ranged from 243 to 399, while *C. plorabunda* varied from 227 to 409.

Only preliminary adult feeding experiments were conducted with *C. plorabunda*. Aphids and sugar water were placed in the cages each day. In most cases the aphids were alive the following day, but occasionally one or two were crushed, apparently by the chrysopid to obtain body juices.

Life history studies in the insectary gave few oviposition records. A number of adults died within a few hours after emergence while others lived only 2 to 3 days. Some mating occurred and as a result a maximum of 14 eggs was laid by *C. plorabunda*. Burke & Martin (1956) stated that *C. oculata* averaged 185.4 eggs from 18 records and *C. plorabunda* 32.0 eggs from 20 records. They used an artificial, high protein food and indicated that it may have increased the egg laying capacity over the previous use of honeydew as food.

Field populations of chrysopids are given in table 4. Data include both adults and larvae and are not identified to species. Smith (1922) found *C. plorabunda* was the most abundant species from early spring to late fall in alfalfa at Manhattan. Surveys indicated two peaks of abundance. The first, August 11, was caused by very high larval counts in two fields. The second peak, September 18, resulted from a population increase and possible influx of adult lacewings into alfalfa, as their abundance coincided with the highest numbers of spotted alfalfa aphids.

Syrphidae.—Table 5 gives the average of 16 records of *Allograpta obliqua* Say. Life history stages were: egg, 2.6 days; larva, 8.4; pupa, 6.5; and the total from the egg to adult, 17.5 days. No visible molting of larvae was noted.

Table 5.—Averages of life history stages of two chrysopids and one syrphid determined by rearing the indicated number of specimens from egg to adult. Manhattan, Kansas, 1958.

Species	No. of Cases	Average Life History in Days						
			Larval Instars			Total Larva	Cocoon[a] or Pupa	Total Egg to Adult
		Egg	1	2	3			
Chrysopa oculata	13	4.0 (4)[b]	3.1 (3–4)	2.8 (2–3)	3.5 (2–4)	9.4 (8–10)	12.6 (12–14)	26.0 (25–27)
Chrysopa plorabunda	17	4.0 (4)	2.9 (2–4)	2.8 (1–3)	3.5 (2–4)	9.1 (8–10)	9.3 (8–10)	22.4 (21–24)
Allograpta obliqua	16	2.6 (2–3)				8.4 (7–10)	6.5 (5–8)	17.5 (16–20)

[a] Includes fourth instar inside spun cocoon, plus pupa.
[b] Range given in parentheses.

Table 6.—Consumption of various sizes of spotted alfalfa aphids by predators. Manhattan, Kansas, 1958.

Species	No. of Cases	Size of Aphid	Average No. of Days Fed	Average Consumed per Predator per Day	Total Aphids Consumed
C. oculata[a]	8	Large	9.6	32.8	2,527
C. plorabunda[a]	8	Large	9.4	34.4	2,604
A. obliqua[a]	12	Large	8.1	33.2	3,269
	2	Medium	9.0	61.1	1,099
	2	Small	10.0	94.4	1,979
Nabis ferus[b]	7	Large	31.6	18.5	3,963
Orius insidiosus[b]	8	Large	25.9	5.4	964
	4	Medium	29.3	10.1	801
	3	Small	31.3	15.3	988

[a] Larvae.
[b] Adults.

It has been reported that with some species larval molting is inconspicuous and probably consists of a continuous shedding of flake-like pieces of the exuvia. Syrphid studies were conducted from June 11 to July 29, 1958, during which time temperatures averaged 74.° F.

Daily average consumption (destruction) of three sizes of aphids by *A. obliqua* during the entire larval period is shown in table 6. In two cases of both medium and small aphids the larvae consumed 61.1 and 94.4 aphids, respectively. In 12 cases the larvae consumed a daily average of 33.2 large aphids. The total of large aphids consumed by individual larvae ranged from 182 to 375. The averages indicate that it required 3 small or 2 medium aphids to equal 1 large aphid.

Specimens reared in the laboratory were never observed to mate or deposit eggs. Gravid females collected in the field readily laid eggs. The highest number recorded was 66 eggs laid by one female. They commonly laid 20 to 30 eggs.

Table 4 gives the average population of all species, both of larvae and adults, collected by field surveys during 1958. Populations were always less than 1.0 per survey date, except September 18, when they averaged 1.0 per field. *A. obliqua* did not comprise more than one-half of the syrphids collected. Adults are rapid fliers and few were taken with a sweep net.

Nabidae.—Life history stages of *Nabis ferus* (Linn.) are given in table 7. A composite of 20 records showed total period from egg deposition to adult averaged 32.6 days. Stages from egg through third instar were almost identical to those reported by Garman & Jewett (1914). Females collected in the field laid eggs that were used for the first group of rearings July 27. The last adult emerged from the second group of eggs October 6, 1958. Temperatures averaged 71° F. during the period. Cold weather developed about mid-September and prolonged development of a few nabids in the fourth instar, but more in the fifth instar.

Table 8.—Consumption of spotted alfalfa aphids by two hemipterous predators. Manhattan, Kansas, 1958.

Species	No. of Cases	Size of Aphid	Average Daily Consumption of Aphids by Instars					Average
			1	2	3	4	5	
Nabis ferus	8	Large	5.1	9.3	13.2	16.9	17.4	12.4
	8	Small	7.8	12.6	18.4	27.4	33.3	19.9
Orius insidiosus	7	Large	4.7	7.9	12.3	15.9	14.9	11.2
	4	Medium	5.9	17.9	28.1	32.0	28.5	22.5
	3	Small	13.9	29.5	34.7	41.0	36.7	31.2

This species was rather difficult to rear. Only 10 of 35 eggs hatched and reached the adult stage. Most mortality occurred during molting from third to final instar in warm as well as in cool weather.

Table 8 shows the average daily consumption or destruction of large and small aphids by the various instars of *N. ferus*. Adult feeding records on large aphids are given in table 6. Seven adults consumed an average of 18.5 per day over an average period of 31.6 days. Daily average consumption of large aphids by fifth-instar nymphs and adults was almost the same. Nielson & Henderson (1959), working with this species, reported males and females destroyed a daily average of 22.1 and 22.6, respectively, over an 8-day period.

Oviposition, by the individuals reared in the insectary, was not observed. Garman & Jewett (1914) on September 19 noted females that had mated in the field showed no inclination to lay eggs. They also reported no eggs were obtained from individuals maturing on that date.

Field survey data for 1958 are given in table 4. Figures shown include immature and adult forms of all species combined. However, *N. ferus* is the most abundant species in the Manhattan, Kansas, area. The averages per survey during 1958 ranged from 6.3 to 16.4, except for July 8, which was lowest at 1.3 nabids per 75 net sweeps.

Anthocoridae.—Table 7 shows the average life history from 14 records of *Orius insidiosus* (Say) reared from egg to adult. The interval averaged about 15 days. Temperatures during this period averaged 80° F.

Garman & Jewett (1914) and Marshall (1930) stated that the fourth instar ranged from 4.36 to 7 days, 2 to 5 days longer than found in this study. Fourteen adults lived an average of 27.8 days and ranged from 5 to 82. This was longer than given by Marshall (1930). Garman & Jewett (1914) found only four instars for *O. insidiosus*.

Table 7.—Averages of life history stages of two hemipterous predators. Manhattan, Kansas, 1958.

Species	No. of Cases	Average Life History in Days							
		Egg	Nymphal Instars					Total Nymph	Total Egg to Adult
			1	2	3	4	5		
Nabis ferus	20	9.2 (9–10)[a]	3.4 (2–5)	2.2 (2–3)	3.4 (2–5)	6.5 (2–12)	10.3 (6–17)	23.5 (15–36)	32.6 (24–45)
Orius insidiosus	14	3.1 (2–4)	2.7 (2–4)	1.7 (1–2)	1.9 (1–3)	1.6 (1–2)	3.6 (3–5)	11.6 (10–14)	14.7 (13–17)

[a] Range given in parentheses.

The average daily consumption or destruction of various sizes of aphids by the nymphs is given in table 8. Averages of small, medium, and large aphids consumed per day during the five instars were 31.2, 22.5, and 11.2, respectively. Average daily consumption by adults is given in table 6. Adults feeding on small, medium, and large aphids, consumed an average of 15.3, 10.1, and 5.4 per day, respectively, indicating that it required 3 small or 2 medium to equal 1 large aphid.

In the feeding process, *O. insidiosus* moved rapidly among the aphids, occasionally stabbing one with its beak. Such aphids often were not fed upon, but were later counted as such because paralysis and death occurred within a few hours after attack.

Two pairs were mated to obtain eggs. One female died 2 days following copulation without ovipositing. The second female deposited 26 eggs in 18 days before dying. Gravid females collected from the field commonly laid 10 to 25 eggs.

Both immatures and adults were combined to obtain survey figures shown in table 4. An average of 15.9 anthocorids were recorded June 27. Counts gradually increased and peaked August 28, then lowered slightly by the last survey date October 2. Insects counted in the field were not identified to species but all representative specimens collected in the field and identified by an authority were *O. insidiosus*.

RELATIVE VALUE OF PREDATORS.—Both high population and high feeding capacity of *H. convergens* ranked it as the most effective of all predators studied (table 9). *C. m. lengi* was second among the coccinellids. *H. g. glacialis* consumed the highest number of aphids but very low populations in the field reduced its effectiveness to fourth place among the coccinellids. The least valuable predator was the coccinellid *O. abdominalis* because of its low numbers in the field. Chrysopids were the second most effective predators. Anthocorids and nabids ranked third and fourth, respectively. Syrphids were the least valuable group because of low field populations. Probably syrphids would be found more numerous and thus more useful than reported here if a more accurate survey method could be devised.

Table 9.—Calculation of theoretical predator effectiveness in consuming large spotted alfalfa aphids in fields near Manhattan, Kansas, 1958.

PREDATORS	AVERAGE		PREDATOR EFFECTIVENESS (POPULATION TIMES CONSUMPTION
	Population of Predators per 75 Sweeps	Daily Aphid Consumption per Predator	
Coccinellidae[a]			
Coleomegilla m. lengi	2.0	46.2	92.4
Cycloneda munda	.3	30.4	9.1
Hippodamia convergens	14.6	89.4	1,305.2
H. g. glacialis	.1	122.1	12.2
H. parenthesis	.7	53.9	37.7
Olla abdominalis	.1	45.0	4.6
Chrysopidae[b]	13.0	33.6	436.8
Syrphidae[b]	.4	33.2	13.3
Nabidae[c]	8.3	15.5	128.7
Anthocoridae[c]	37.3	8.3	309.6

[a] Only adult form evaluated.
[b] Only larval form evaluated.
[c] Both nymphs and adults evaluated.

Predator effectiveness of coccinellids as given in table 9 may be low since larvae were not included in the calculations. Chrysopids would probably be slightly lower in relative effectiveness than shown, since calculations included both immature individuals and adults from surveys, however, *C. oculata* would offset this to some degree as the adult consumes a considerable number of aphids (Burke & Martin 1956).

Goodarzy & Davis (1958) in Utah, using this method of evaluating predators of the spotted alfalfa aphid, also reported that *H. convergens* was the most important of all predators. They found *Chrysopa*, *Orius* and *Nabis* were second, third, and sixth most effective, respectively. All stages of *H. convergens* consumed an average of only 29.5 mature aphids each day whereas in the present study all stages destroyed 67.2 large aphids. Goodarzy & Davis (1958) and Nielson & Henderson (1959) in similar studies of adult *H. convergens* feeding on the mature spotted alfalfa aphid reported an average consumption of 32.9 and 96.7 aphids per day, respectively.

C. plorabunda averaged only 15.2 aphids daily (Goodarzy & Davis 1958) whereas in our study it consumed 34.4 aphids per day during the larval period.

Evaluation of effectiveness of predators is complex and the values obtained in our study are only relative and apply only to the fields and years studied. Several factors such as periodic mowing of alfalfa, fluctuating numbers of aphids and other insect hosts, heavy rainfall, climatic and environmental conditions influenced the predator populations. Since these predators feed on insects other than spotted alfalfa aphids, a study to determine their preference for competing food hosts is needed to evaluate their effectiveness more accurately.

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Surveys for Nosema Disease of Honey Bees in California[1]

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ABSTRACT

Two surveys were made of the major beekeeping areas of California to determine the distribution and incidence, in apiaries and individual colonies, of nosema disease, an infection of the adult honey bee, *Apis mellifera* L., by the protozoan parasite, *Nosema apis* Zander. A comparison was made of different sampling techniques using live and dead bees from inside and outside the hives and a mechanical sampler was constructed for collecting field bees from hive entrances. In 1955, 13.9% of the colonies sampled were infected and the disease was present in all areas of the state. In 1958, 85.4% of the colonies in four apiaries sampled were infected. From 0% to 63% of the field bees in individual colonies were infected. Infection decreased during the summer but was present into October. Environment did not seem to affect the level of infection among the colonies.

Nosema disease is the common name for the infection of the adult honey bee, *Apis mellifera* L., by the protozoan parasite, *Nosema apis* Zander. The organism was first recognized in Europe in 1857 when Dönhoff observed the spores in the stomachs of bees. In 1909 Zander described the organism and proved it was the cause of an endemic disease of honey bees. Since then it has been found in beekeeping areas throughout the world wherever a search has been made for it.

The disease varies in severity in different areas and from year to year. This characteristic has been responsible for a wide range of opinions about its economic importance. Research in the United States by Farrar (1942, 1947, 1954) and in Canada by Jamieson (1955) has shown that nosema infection contributes to premature supersedure of queens, abnormal winter losses, and reduced honey yields. However, information is lacking on the incidence and severity of the disease in most areas of the United States because surveys for it have not been made.

In 1921 and 1922 samples of bees from all parts of the United States were examined by the U. S. Department of Agriculture Bee Culture Laboratory at Beltsville, Maryland, for the presence of adult honey bee diseases (Phillips 1922, 1923). Of the 39 samples received from California, 10 contained spores of *Nosema apis* and another was possibly infected. Two other samples from California were diagnosed as infected with nosema in 1929 (Todd 1929). In spite of this early evidence that nosema was present in California, it was commonly believed until recently that the disease did not occur there (Krebs 1947). According to Eckert (1954a, b) and Eckert & Laidlaw (1957) nosema is of little economic importance in California except in the San Francisco Bay area and along the coast.

The work reported here was done with four objectives: (1) to compare the amounts of nosema detected by different types of samples, (2) to determine the distribution and incidence of nosema in apiaries and individual colonies, (3) to determine the seasonal trend of the disease in areas with hot summer temperatures and (4) to observe any effects of environment upon the distribution and incidence of the disease.

METHODS AND MATERIALS.—The method of sampling colonies for nosema will obviously affect the results of a survey for the disease. The method best suited for determining the prevalence of infection in an apiary may not be the best for determining the percentage infection within a colony. Since initial survey efforts were to be concerned only with the infection within apiaries, a comparison of sampling methods was necessary. Betts (1951) has suggested that dead or dying bees give a good indication of the prevalence of nosema. Samples of live and dead bees were collected from 19 nuclei (small colonies) and six large colonies in February 1955. The infection in 10 dead bees from in front of the hives was compared with that in 10 bees from the top of the colony cluster.

The abdomens of the bees from each sample were macerated with 1 to 2 ml. of water using a heavy glass rod and a shell vial. A drop of the material was examined under a compound microscope at 440× magnification for spores of *Nosema apis*. The same technique was used in all subsequent sampling.

The comparison of types of samples was continued in a queen-rearing apiary near Davis, California. Samples of 10 bees were collected from inside and outside the hives. Pieces of white cotton sheeting 12×24 inches were placed in front of each nucleus as a collection spot for the dead bees ejected from the colony. The cloths were held in place by a number of small rocks which also served to keep the dead bees from blowing away. Once a month, from March to October 1955, samples were collected from the nuclei and examined for nosema. All dead bees were removed from the cloths at each sampling. These samples were used also to detect the seasonal trend of the disease under the warm summer conditions prevailing at Davis.

The first survey for incidence and distribution of nosema was carried out from February to April 1955. It was conducted by collecting 10 or more dead bees from in front of every 10th hive in the apiary being checked. With the help of the apiary inspectors of the counties, samples were collected in 82 apiaries belonging to 45 beekeepers. Eleven counties were represented, from Shasta County in the north to Imperial County in the south. At each location notes were made of the type of terrain, exposure, and sources of water for the colonies so that information might be gained on the effect of environment upon the distribution and incidence of nosema.

This series of samples from many areas of California showed that nosema is widespread in the State but offered no hint of the degree of infection within the colonies. A second survey was initiated in February 1958 to obtain figures on the percentage of nosema-infected field bees in colonies in several of the major beekeeping regions of the State.

In order to catch field bees in sufficient numbers for the samples, a copy of a hand-operated bee sampler designed by Bailey (1956) was constructed. Art Freeman

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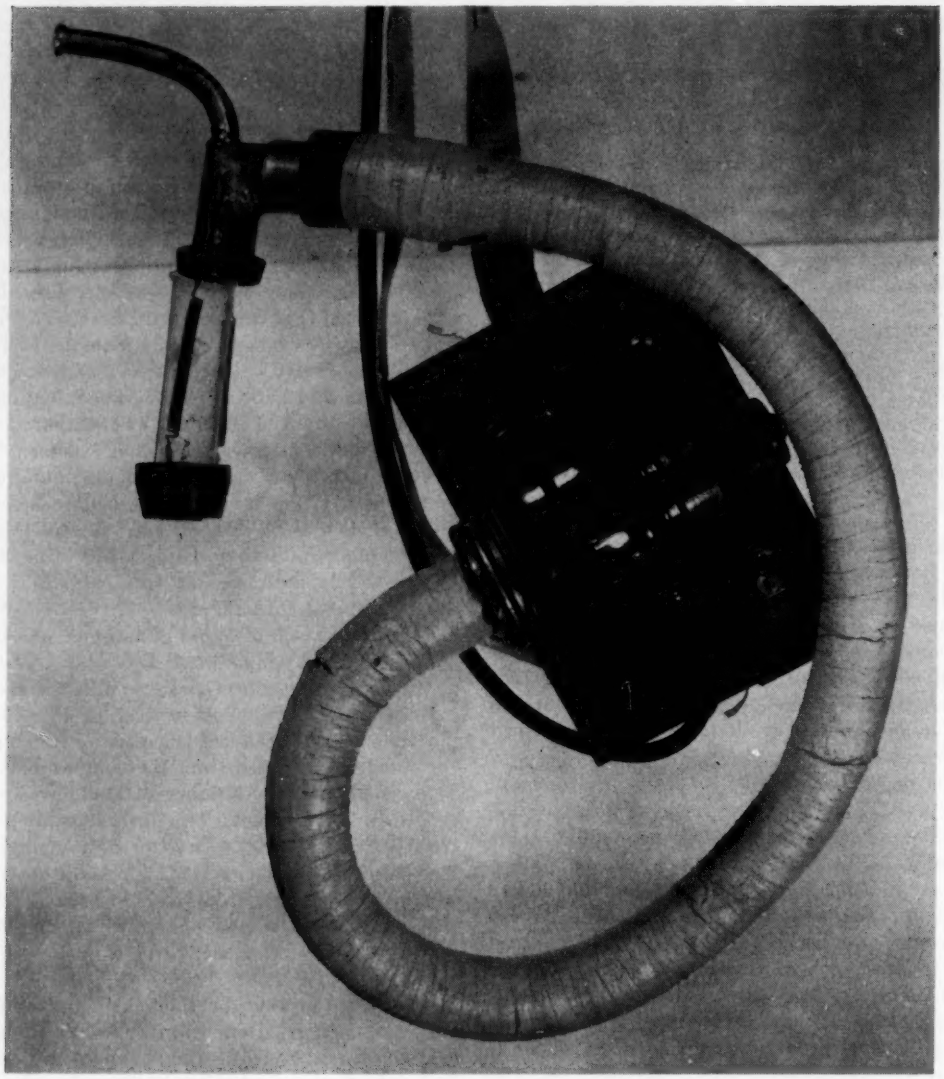

FIG. 1.—Battery-operated bee sampler. (Photograph courtesy of California Department of Agriculture.)

of the California Department of Agriculture assisted in mechanizing the sampler, using a 6-volt motor and squirrel-cage blower to produce the necessary suction (figure 1). The head of the sampler was connected to the intake side of the blower by a piece of flexible vacuum cleaner hose. In the apiary, the sampler was connected to a 12-volt automobile battery by a heavy duty extension cord. The unit produced greater suction when operated on the higher voltage and did so without being damaged, probably because it was used only intermittently for short periods. When sampling an apiary, several colonies were closed at one time, with strips of burlap pushed into the entrances. One man closed and opened the colonies while the other operated the sampler.

Of necessity, the size of sample and method of analysis used for field bees required a compromise because they had to give a reasonable level of significance for the colonies with large field populations yet be easily handled with limited laboratory help. The method of analysis selected required samples of 50 to 100 bees. Mass samples of 10 bees were examined until one was found containing *Nosema apis* spores or until five were found negative. When an infected sample was found, 50 bees from the same colony were examined individually for infection by macerating the abdomen of each. When possible, samples were collected from 25 colonies in each apiary. In two of the apiaries fewer colonies were sampled because too few bees returned to the hives to provide an adequate sample. This condition appeared to result from low colony populations and the disruption of normal flight activity while sampling the apiary.

RESULTS.—*Type of Sample.*—The initial samples of live and dead bees from 19 nuclei and six large colonies revealed nosema infection in four samples of dead bees but in only one sample of live bees. Similar samples from the queen-rearing apiary near Davis also contained more nosema infection in the dead bees collected from outside the colony (table 1). Spores were found in 25 samples of

Table 1.—Nosema infection in samples of live bees from inside and dead bees outside of honey bee nuclei at Davis, California, March–October, 1955.

Nucleus No.	Source of Sample	Month Sampled								Total Infections
		Mar.	Apr.	May[a]	June	July	Aug.[a]	Sept.[a]	Oct.[a]	
1	inside	−	−	−	−	−	−	+	−	1
	outside	−	−	+	−	−	−	+	−	2
2	inside	+	−	−	−	−	−	−	−	1
	outside	−	−	+	−	+	+	−	−	3
3	inside	−	−	−	−	−	−	−	−	0
	outside	+	−	−	−	+	+	−	−	3
4	inside	−	−	−	+	−	+	+	+	4
	outside	+	−	no bees	+	−	−	+	−	3
5	inside	−	−	+	−	−	−	−	−	1
	outside	+	−	+	+	−	−	−	−	3
6	inside	−	+	+	−	−	−	−	−	2
	outside	+	+	−	+	−	+	−	−	4
7	inside	−	−	+	−	−	+	−	−	2
	outside	−	−	−	−	−	−	−	−	0
8	inside	−	−	−	+	−	−	−	−	1
	outside	−	+	+	+	−	−	−	−	3
9	inside	+	+	+	−	−	−	−	−	3
	outside	−	+	no bees	−	−	−	−	−	1
10	inside	+	+	−	−	−	+	−	−	3
	outside	−	−	+	+	−	−	−	−	2
11	inside	+	−	−	−	−	−	−	−	1
	outside	−	+	−	−	−	−	−	−	1
12	inside	+	nucleus discontinued							1
	outside	−								0
Totals	inside	5	3	4	2	0	3	2	1	20
	outside	4	4	5	5	2	3	2	0	25

[a] Some outside samples for these months contained less than 10 bees.

dead bees and in only 20 samples of live bees from within the nuclei.

Distribution and Severity of Infection.—The first survey showed that nosema was present in about 14% of the colonies throughout the state (table 2). Infected colonies were found in every county sampled except Colusa. Additional samples taken there the next year contained infected bees.

In the 1958 survey 85.4% of the colonies in four apiaries showed nosema infection. In two of these every colony was infected. The number of colonies at eight infection levels (0% to 70%) for these apiaries is given in table 3. The Orange County apiary had a mean percentage of infection within the colonies of 28.4, with a range from 12% to 63%. The Yolo County apiary, also showing infection in every colony, had a mean percentage infection of 15.4 within the colonies, ranging from 2% to 57.1%. The average percentage of colonies infected was far higher than the 14.7% found among full colonies in the first survey in 1955.

Table 2.—Nosema infection among honey bee nuclei and full-sized colonies in apiaries in 11 California counties, 1955.

County	Date 1955	Number of Samples		Percentage Infected		
		Nuclei	Colonies	Nuclei	Colonies	All Samples
Shasta	2/9	125	25	7.2	4.0	6.7
Tehama	3/24	9	41	9.7	0	8.0
Butte	3/8	0	63	—	22.2	22.2
Glenn	3/4	0	51	—	5.9	5.9
Colusa	3/11	1	76	0	0	0
Yolo	3/3	108	22	12.0	22.7	13.8
Sacramento	2/17	15	4	73.3	75.0	73.8
Solano	2/19	1	4	0	25.0	20.0
Tulare	4/21	0	19	—	21.1	21.1
Riverside	3/28	0	23	—	73.9	73.9
Imperial	3/30	0	59	—	8.5	8.5
Total samples		259	387			
Total percentage infected				12.7	14.7	13.9

Table 3.—Nosema infection within honey bee colonies in apiaries in four counties of California, 1958.

Percentage Infection Within Colony	Number of Colonies			
	Shasta 5/1	Orange 5/7	Yolo 5/16	Tulare 6/11
0	3	0	0	10
1–10	7	0	8	10
11–20	3	6	13	1
21–30	5	5	2	1
31–40	5	4	1	0
41–50	0	0	0	0
51–60	2	0	1	0
61–70	0	2	0	0
	25	17	25	22

Seasonal trend of infection.—It is often stated that nosema disappears in areas with hot summers such as those at Davis, California. The samples from nuclei during the eight months of March to October revealed that infection did decrease during the period but was present all that time (table 1). A maximum of five nuclei was found infected at any one sampling period, yet, during the course of the sampling, nosema was present in samples from *every* nucleus.

DISCUSSION.—The figures for nosema infection from the two surveys reported here are not directly comparable with many of the figures reported as a result of other types of surveys. Such surveys are frequently based on samples received routinely for diagnosis or in response to an appeal for samples of sick or abnormal bees. For example, Murray (1952) reported that 3% of the samples diagnosed routinely over a 3-year period in Scotland were infected with nosema.

Jamieson (1945) examined samples from all areas of Canada sent to him in response to an appeal in beekeeping magazines for abnormal bees and prematurely superseded queens. He found *Nosema* spores in 58% of the samples of workers and in 52.3% of the queens. The latter figure is high but not surprising in view of the relationship between nosema disease and premature supersedure established by Farrar (1947). Betts (1951) states that one queen out of three in colonies having nosema may be infected.

During the season in which the initial California survey was conducted, routine examinations were made for nosema in samples of bees received in Sacramento for disease diagnosis or pesticide analysis. Of 35 samples, 16 (45.7%) were infected. This percentage is more than three times as great as that found in the survey and helps to emphasize the variations between field samples and those sent in.

Farrar's limited survey (1947) using random sampling of colonies in Mississippi, Louisiana, and Alabama was made in a manner similar to the first California survey. He found 51% of the colonies and 65% of the nuclei infected with nosema. Ellis & Wilson (1957) reported a 33.5% infection among colonies in Mississippi in May 1956.

These figures, while difficult to compare and interpret in relation to the seriousness of the disease, demonstrate the wide variation in incidence of nosema from year to year and from place to place. Surveys like those of Murray (1952) and Jamieson (1945) can be expected to indicate a higher incidence of nosema than random field surveys because the samples are usually biased by being drawn from abnormal colonies. The above-mentioned samples submitted to Sacramento provide another good illustration of this point.

One objective of the initial survey was to determine any relation between environmental factors of the colonies and the incidence of nosema. A record was made at each location of the colony exposure, amount of sunlight, source of water and amount of moisture in the area. There was no indication that nosema infection was influenced by the immediate environment. Apiaries in damp, shaded locations along the Sacramento River had no more infected colonies than those in sunny, dry desert areas.

Colony conditions were quite different during the two surveys because of the extremely wet weather prior to the second survey. Many colonies ran short of pollen and honey and were confined to their hives for long periods. The resulting interruption of brood rearing retarded the increase in size of the colonies. These colony conditions were favorable for an increase in the incidence of nosema and probably kept it at a higher level into the month of May. However, by the second week in June when samples from Tulare County were examined, the normal seasonal reduction in infection had probably begun.

During both surveys the commonly described symptoms of nosema were not noticeable. In many locations mice and birds had carried away the dead bees. Even where they did not, there was little or no indication that the colonies were infected. When the random sampling failed to include colonies showing large numbers of dead bees, extra samples were taken. Most of them were not infected.

The inadequacy of 10-bee samples is evident in the data from the monthly samples of the queen-rearing nuclei shown in table 2. Samples of bees from inside nucleus number 3 did not reveal any infection during the 8 months. Over the same period, three samples from outside the nucleus were infected. Nucleus number 7 appeared to be free of infection in the eight samples from outside the hive, yet two samples of bees from within the nucleus contained spores. The incidence of infection among colonies as determined with the 10-bee samples was probably low. Even a corrected figure would not approach the 85.4% infection found in the second survey. The latter figure probably reflects the influence of colony conditions upon nosema rather than the inaccuracy of the smaller samples. A single sample of at least 25 bees, or several samples of 10 bees, would reduce the error with little additional work.

The surveys show that incidence of nosema in California is as great as in any other state or country. California beekeeping is characterized by queen-rearing, use of nuclei, and frequent long-distance moving. These practices are probably conducive to an increase in the nosema infection as suggested by Bailey (1955a, b). Nosema control would probably be profitable, therefore, in beekeeping operations especially favorable to the disease. One of these is the production of package bees and queens. Another is honey production in the sage and buckwheat areas of southern California where good nectar flows occur soon after wet weather favorable to increased infection.

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Cumulative Effect of Sublethal Doses of Myristyl Chloroacetate on a Highly DDT-Resistant Strain of *Musca domestica* L.[1]

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Abstract

Female house flies (*Musca domestica* L.) have been exposed, by contact, to repeated sublethal doses of myristyl chloroacetate, at different time intervals. It has been shown that there is an accumulation of the toxic effects of this substance in *M. domestica*, and that the accumulation is correlated with the length of time intervals. Detoxification probably results from at least two regeneration processes which proceed at different speeds.

Several studies on the cumulative effect of toxic substances on insect are reported in the literature, which has been reviewed up to 1952 by Beard. Recently, Hoffman *et al.* (1951) have observed that by exposing house flies (*Musca domestica* L.) periodically to low concentrations of DDT by contact on glass, a cumulative effect was shown and that the number of survivors was greater for longer intervals between exposures, while, as determined by employing labeled DDT, the total amounts of insecticide absorbed were similar in two lots, one of which exposed continuously and the other intermittently. Beard (1952) studying the effect of sublethal doses of toxicants on the susceptibility of *Oncopeltus fasciatus* adults and *Galleria mellonella* larvae, found that by administering two unit doses at different times, arsenic appeared to be eliminated, by *Galleria* larvae, but was additive in *Oncopeltus;* both DDT and pyrethrum showed some sensitizing activity, and in general were more additive.

A study by Bliss & Beard (1953) on the static and dynamic variation in the response of *Oncopeltus fasciatus* to sublethal doses of CO_2 and N_2O showed that with repeated exposures of 30 seconds to either gas and rest intervals of less than 30 minutes between the righting response and the next exposure to gas, the time for the righting response increased progressively. Hadaway (1956), studying the effects of repeated sublethal doses of various insecticides by topical application to a susceptible strain of *Musca domestica*, found that practically no cumulative effect could be shown with BHC (gamma isomer), while with DDT, dieldrin and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothiate) a cumulative effect, increasing in the order named, was observed.

McCuaig & Sawyer (1957) found that following repeated topical applications of sublethal doses of dinitro-*o*-cresol on *Locusta migratoria* and *L. gregaria* no cumulative effect was evidenced.

The present paper reports research on the cumulative effect of myristyl chloroacetate as representative of a group of substances (iodo-, bromo- and chloroacetic acids and their derivatives[3]) studied in the last few years by our group (Bettini & Boccacci 1958, Boccacci & Bettini 1956, Bettini *et al.* 1958a, 1958b, Boccacci *et al.* 1959). These toxic substances act by blocking the thiol vital functions (Barron 1951) ($R \cdot SH + ClCH_2 \cdot COOH = R \cdot SCH_2 \cdot COOH + HCl$). Since this reaction is an irreversible one, by exposing the insect to repeated sublethal doses of a substance belonging to this group, a cumulative effect should be shown. In the present paper by the term "cumulative effect" we do not mean the phenomenon of true accumulation of a toxic unmetabolized substance in the insect, but rather an accumulation of its toxic effects [dynamic or physiological accumulation (Adami 1947)].

As demonstrated by earlier work (Bettini *et al.* 1958b) the halogen-containing thiol alkylating agents show an equal degree of toxicity to susceptible as well as to resistant flies. Therefore, it seemed to be of interest to use a resistant strain of house flies in the work reported herein.

Materials and Methods.—Biological material: female house flies used were 3 days old, from a laboratory strain (K_1), originating from Geigy, Basel, highly resistant to DDT and resistant to other chlorinated hydrocarbons, bred according to a standard technique. Product employed: myristyl chloroacetate (b.p. 150° to 153°/0.5 mm.).

The flies have been exposed following a method described elsewhere (Bettini *et al.* 1958a) according to which the insects have been kept in contact with the toxicant in petri dishes (covered by nets) for 30 minutes. During the time intervals between treatments the flies have been maintained at room temperature in clean cages and fed sugar and water.

The exposures to the toxicant have been carried out at intervals of 2, 10, 24, 72, and 120 hours. Knockdown has been scored at 24 hours from each exposure (knockdown brought about by the group of substances, to which myristyl chloroacetate belongs, is an irreversible phenomenon; therefore, all knocked down flies were considered dead). For each exposure different petri dishes, treated 24 hours ahead, have been used.

Since the experiments lasted in some cases several days, the LD_{50} variations according to age were determined. Abbott's formula has been applied for correction of natural mortality.

Results and Conclusions.—As may be observed from table 1, the LD_{50} values are constant until the fifth day of age.

In figure 1 are shown the results obtained by exposing flies to repeated treatments with a dose of 0.30 gm./m.^{2}, at different time intervals. It may be readily observed from figure 1 that, as the time intervals increased, the mortality for an equal number of exposures decreased progressively. In fact, at the second exposure, the mortality reached 78% for a 2-hour interval, while only 5% for a 120-hour interval.

In the trials at 72- and 120-hour intervals, which lasted more than 5 days, the greater susceptibility of flies, due to age, was accounted for and a correct value of mortality (M) was calculated according to the formula $M = M_a - (M_b - M_c)$, where M_a represented the mortality of flies more than 5 days old, at the second or successive expo-

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[2] The authors wish to thank Mr. G. Pierdominici for helpful assistance.

[3] Halogen containing thiol alkylating agents.

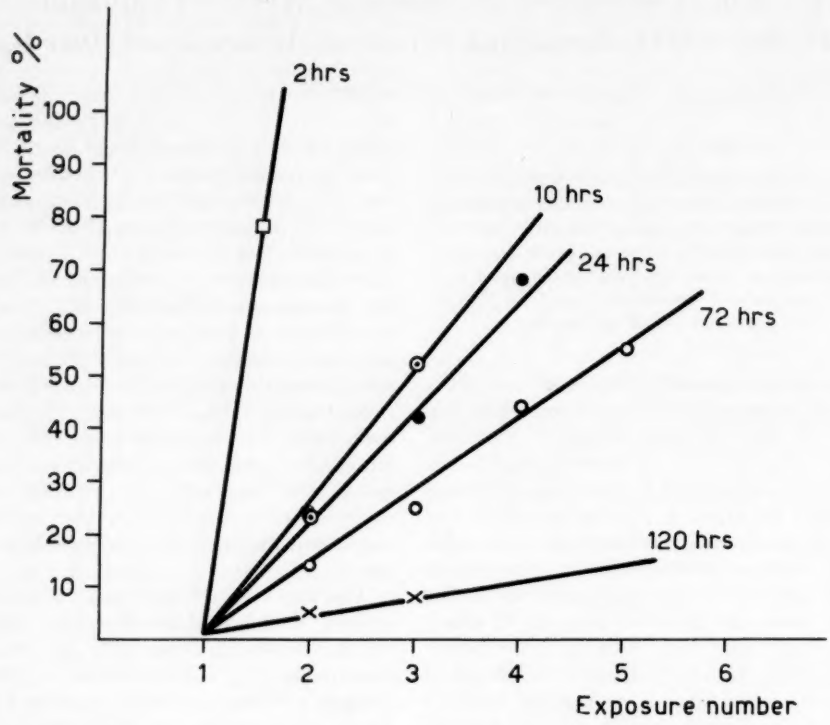

FIG. 1.—Cumulative mortality of female flies repeatedly exposed to petri dishes treated with 0.30 gm./m.² of myristyl chloroacetate. Time intervals between exposures, 2, 10, 24, 72, and 120 hours. Temperature 24° to 25° C.; weight 22 to 24 mg./fly. Groups of 300 flies, or more, each.

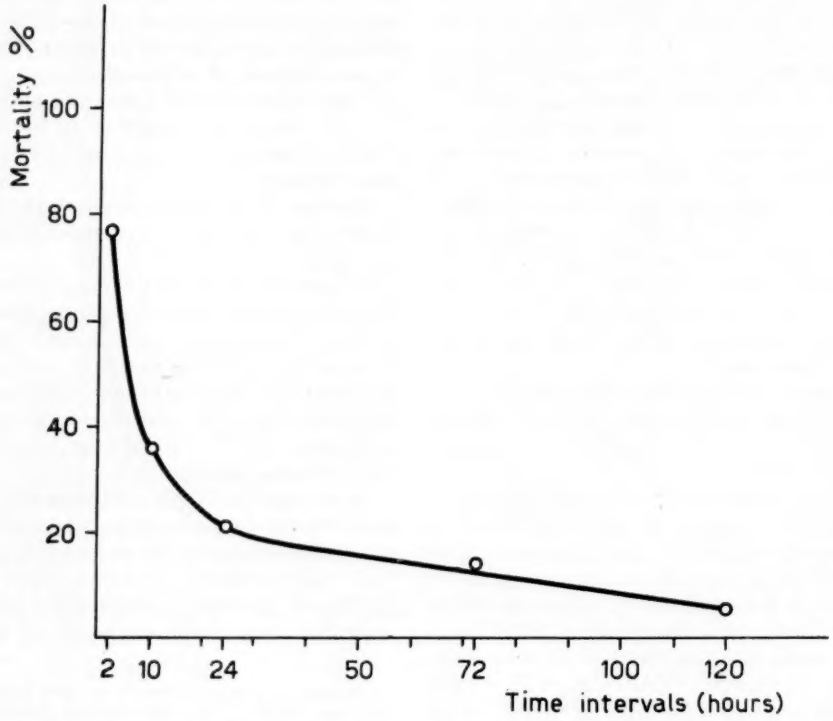

FIG. 2.—Per cent mortality following the second exposure to petri dishes treated with 0.30 gm./m.² of myristyl chloroacetate, at different time intervals between the two exposures.

Table 1.—Variations in LD_{50} values, according to age, in female house flies exposed to petri dishes treated with myristyl chloroacetate.[a]

Age (days)	1	2	5	7	9	11	13	15
LD_{50} (gm/m².)	0.44	0.44	0.44	0.40	0.40	0.37	0.34	0.33
Natural mortality (%)	0	3	4	4	1	2	7	13

[a] Length of contact: 30 minutes. Weight 21 to 23 mg./female. Temperature 20°–24° C. The LD_{50} values were determined by using 100 flies/each dose.

sures; M_b the mortality of flies of corresponding ages, but at the first treatment; M_c the mortality of flies 3 days old exposed to a single treatment.

From a study of the data presented in this paper it may be deduced that an evident accumulation of toxic effects is shown by *Musca domestica* exposed to repeated sublethal doses of myristyl chloroacetate. Such an accumulation is correlated with the length of time intervals between exposures as expressed in figure 2. From an examination of this graph it seems probable that detoxification results from at least two regeneration processes which proceed at different speeds.

Detoxification, therefore, because of the peculiar mode of action proper of this group of products (to which myristyl chloroacetate belongs), should result from the regeneration of the substances bearing SH-group blocked by the toxicant. It is reasonable to assume that among these substances there are some having a low regeneration time (*e.g.*, cysteine and glutathion) and others having a much higher regeneration time (*e.g.*, proteins).

From figure 2 it also appears that the time interval between exposures should last, for a complete recovery, about 150 hours.

The cumulative effect of myristyl chloroacetate, thus shown, would be expected to represent a property common to all "halogen-containing thiol alkylating agents" and confers to this group of substances a probable insecticidal value of some interest even though their toxicity is of a low order of magnitude.

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Colonization and Production of a Dieldrin-Resistant Strain of *Anopheles quadrimaculatus* Say[1]

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ABSTRACT

A dieldrin-resistant field strain of *Anopheles quadrimaculatus* Say from Cleveland, Mississippi, has been established under insectary production. Three-day-old females show high dieldrin resistance; 60-minute exposures to 0.8% through 8% dieldrin-Risella oil papers result in less than 10% mortality in 24 hours, while 480-minute exposures to 4% papers killed only 50%. Susceptible females are killed by 60-minute exposures to 1.6% dieldrin-Risella oil papers.

Initial attempts in 1956 and 1957 to colonize a dieldrin-resistant field strain of *Anopheles quadrimaculatus* Say from Cleveland, Mississippi were unsuccessful. Field-collected blood-engorged females produced eggs which were shipped airmail to the laboratory and reared to adults by standard insectary procedures. Twenty-four-hour mortality values for the resultant 3-day-old adult females with 30-minute exposures to residual deposits of 25, 100, and 200 mg. of dieldrin per square foot averaged 5%, a level comparable to that of the field adults (Mathis *et al.* 1956). Only an occasional insectary-reared female fed on the hare or human hosts provided, and the few eggs laid gave rise only to susceptible individuals.

Studies were continued in 1958. Since the biting rate of inseminated females was found to be about three times greater than that of unmated females (Terzian & Stahler 1949), mating studies were conducted starting with a susceptible laboratory strain. Adult males and females were separated when less than 24 hours old and kept apart

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[2] From the Communicable Disease Center, Bureau of State Services, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia.

until 3 days old. At that time they were recombined in a small (18″×18″×18″) cage 3 hours before sunset. The first observed activity of the mating cycle, waving of the antennae by the males, occurred 4 minutes before sunset (meridian lighting—11 foot-candles). At sunset the males began waving their hind legs vertically (meridian lighting —8 foot-candles). Shortly after this, the males swarmed in the center of the cage and the females ceased resting on the sides and top of the cage, and entered the swarm. Mating in flight commenced 5 minutes after sunset (meridian lighting—4.8 foot-candles). The copulating pairs moved about the cage, often falling to the bottom. Copulation was rapid, not lasting more than 10 or 15 seconds with the mating pair oriented head to head, the male underneath. At the peak of mating, as many as 12 pairs were observed per minute out of a total of 120 females and 140 males in the cage. Mating continued until the meridian lighting was less than 0.01 foot-candle. In a repeated study essentially the same results were obtained.

When 3-day-old adults of the resistant strain were compared in separate cages with those of the susceptible strain, the latter strain mated shortly after sunset; however, no mating was observed in the resistant strain. Although the males reacted in about the same manner as the susceptible males, the females did not respond but instead remained on the sides and top of the cage. These studies were repeated several times. Although the susceptible strain mated freely each time, the resistant strain has never been observed mating.

The resistant adults were removed after each mating experiment and placed in a large (3′×3′×3′) cage on a screened porch. Pupae reared from eggs received from Cleveland, Mississippi were added to this cage. Since one of the best field sources of the blood-engorged females had been pig houses, and since Horsfall (1955) notes that "Pigs, dogs, and, to a varying extent, fowls were often chosen as sources for blood meals," a young, newly-weaned pig was tried as a host. The pig was placed in the large colony cage nightly and an oviposition pan was provided. Viable eggs were produced.

The pig was somewhat unsatisfactory as a host (a) since adult mosquitoes escaped when the pig was introduced and when it was removed from the colony cage through a cloth sleeve and (b) since rapid weight gain necessitated a new host animal approximately every 6 weeks. After 4 months, further attempts were made to use a hare as the host animal. A special cage was used to present both the pig and the hare to the females. It was observed that the females fed on the animal that was presented first, thus a hare was adopted successfully as the only source of blood. The females engorged readily and no decrease in egg production occurred.

Since initially only 10% of the larvae completed pupation, relatively few adults were available for determining dieldrin susceptibility of the insectary-produced resistant strain. Over several generations, however, 3-day-old females of the resistant and the susceptible strains were compared in the laboratory by a method developed for resistance measurements (Mathis *et al.* 1959) using 60-minute exposures to various dieldrin-Risella oil-impregnated papers prepared by the World Health Organization. Results (table 1) showed that dieldrin resistance had been retained by the strain since they were only slightly affected by the 1.6% dieldrin-Risella oil papers, which killed all of the susceptible strain.

Since the mortalities of the 3-day-old resistant females (table 1) were less than 10% with 60-minute exposures for 0.4% through 8% dieldrin, longer exposure periods were tried. Accordingly, 3-day-old resistant females were exposed to 4% dieldrin-Risella oil papers for periods ranging from 60 to 480 minutes (table 2). The maximum exposure killed about half of the females exposed, again indicating high resistance.

Further tests were conducted with resistant adults less than 24 hours old. Adult males and females, exposed separately to various dieldrin-Risella oil-impregnated papers for 60 minutes, indicated that only about half of the adults succumbed at this age (table 3). A series of exposures, varying from 60 to 300 minutes, were conducted also with 4% dieldrin-Risella oil papers to determine the effect of exposure time upon mortality (table 4). Since a marked difference in resistance existed between 3-day-old and less-than-1-day-old females, 1- and 2-day-old fe-

Table 1.—Per cent mortality of 3-day-old female *Anopheles quadrimaculatus* from 60-minute exposures to various dieldrin-Risella oil-impregnated papers.

Per Cent Dieldrin-Risella Oil	24 Hour Mortality (%)	
	Susceptible Strain	Resistant Strain
8.0	—	6 (233)[a]
4.0	—	6 (208)
3.2	—	8 (275)
1.6	100 (184)	7 (283)
0.8	73 (179)	3 (266)
0.4	10 (188)	3 (277)
Control	0 (107)	3 (274)

[a] Numbers of individuals tested are shown in parentheses.

Table 2.—Per cent mortality of 3-day-old resistant female *Anopheles quadrimaculatus* exposed to 4% dieldrin-Risella oil-impregnated papers for varying perods.

Exposure Period (Minutes)	24-Hour Mortality (%)
60	4 (744)[a]
120	11 (463)
240	25 (405)
480	50 (451)
Control	1 (512)

[a] Numbers of individuals tested are shown in parentheses.

Table 3.—Per cent mortality of less-than-24-hour-old resistant *Anopheles quadrimaculatus* from 60-minute exposures to various dieldrin-Risella oil-impregnated papers.

Per Cent Dieldrin-Risella Oil	24-Hour Mortality (%)	
	Males	Females
8.0	—	47 (147)[a]
4.0	61 (49)	36 (199)
3.2	58 (52)	50 (264)
1.6	42 (50)	34 (287)
0.8	35 (54)	28 (204)
0.4	—	20 (126)
Control	18 (45)	3 (264)

[a] Numbers of individuals tested are shown in parentheses.

Table 4.—Per cent mortality of less-than-24-hour-old resistant *Anopheles quadrimaculatus* exposed to 4% dieldrin-Risella oil-impregnated papers for varying periods.

Exposure Period (Minutes)	24-Hour Mortality (%)	
	Males	Females
60	48 (160)[a]	39 (263)
120	70 (161)	55 (125)
180	77 (159)	76 (431)
240	—	78 (252)
300	—	83 (132)
Control	5 (161)	3 (210)

[a] Numbers of individuals tested are shown in parentheses.

males also were exposed separately to dieldrin-Risella oil papers for 60 minutes (table 5). These tests showed that the response of the 1- and 2-day-old females was similar to that of the 3-day-old females.

One test has been run to determine the DDT-susceptibility of the strain. Three-day-old females of both the dieldrin-resistant and the susceptible strains, exposed to DDT-Risella oil papers for 60 minutes, showed quite comparable mortalities (table 6). The resistant strain is presently maintained under no selection, and, with improved rearing methods, about 20% of the larvae reach the pupal stage. The strain is now considered as established under insectary production.

Table 5.—Per cent mortality of 1-, 2-, and 3-day-old resistant female *Anopheles quadrimaculatus* exposed to various dieldrin-Risella oil impregnated papers for 60 minutes.

Per Cent Dieldrin-Risella Oil	24-Hour Mortality (%)		
	1-Day Old	2-Day Old	3-Day Old
8.0	7 (144)[a]	5 (200)	6 (233)
4.0	7 (126)	1 (202)	6 (208)
3.2	10 (123)	2 (206)	8 (275)
1.6	11 (128)	1 (203)	7 (283)
0.8	16 (131)	1 (193)	3 (266)
0.4	9 (132)	0 (179)	3 (277)
Control	1 (149)	1 (202)	3 (274)

[a] Numbers of individuals in test are shown in parentheses.

Table 6.—Per cent mortality of 3-day-old female *Anopheles quadrimaculatus* from 60-minute exposures to DDT-Risella oil-impregnated papers.

Per Cent DDT-Risella Oil	24-Hour Mortality (%)	
	Susceptible Strain	Resistant Strain
4	89 (75)[a]	82 (77)
3	—	67 (106)
2	22 (75)	67 (76)
1	0 (77)	16 (72)
Control	0 (75)	1 (78)

[a] Numbers of individuals tested are shown in parentheses.

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Laboratory Studies on the Effectiveness of Chipman R-6199 against Some Cotton Pests[1]

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Abstract

Chipman R-6199, the monohydrogen oxalate of *O,O*-diethyl *S*-(2-diethylamino)-ethyl phosphorothioate, was tested against insects introduced on potted cotton plants held in the laboratory. It was as effective as endrin against second- and third-instar larvae of the salt-marsh caterpillar (*Estigmene acrea* (Drury)), cabbage looper (*Trichoplusia ni* (Hbn.)), and cotton leafworm (*Alabama argillacea* (Hbn.)). At 5 mg. per plant it was also effective against adults of the boll weevil (*Anthonomus grandis* Boh.) and pink bollworm (*Pectinophora gossypiella* (Saund.)), but the residual effect was short-lived. At the same rate it gave 100% kill of the cotton aphid (*Aphis gossypii* Glov.) for 25 days and of the two-spotted spider mite (*Tetranychus telarius* (L.)) and nymphs of the thrips (*Hercothrips phaseoli* (Hood)) for at least 48 days. In fresh deposits R-6199 was more toxic than DDT to pink bollworm moths. Results with a 1:3 mixture of R-6199 and DDT indicated slight additive action. Residues on leaf surfaces decreased rapidly in the first 2 days, but those inside the leaves increased gradually. Detached leaves were also found to absorb the chemical. Translocation to new terminal leaves was slow.

Chipman R-6199, the monohydrogen oxalate salt of *O,O*-diethyl *S*-(2-diethylamino)-ethyl phosphorothioate, has been reported to be effective against several cotton pests (U. S. Agricultural Research Service 1958). It gave good control of several species of spider mites and the cotton aphid (*Aphis gossypii* Glov.) when used as a spray, and it killed the first and second instars of the salt-marsh caterpillar (*Estigmene acrea* (Drury)) feeding on plants

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[2] In cooperation with the Texas Agricultural Experiment Station.

Brian Ashworth, of the Plant Protection Ltd., England, made the residue analyses at the laboratories of Chipman Chemical Company, Bound Brook, N. J

topically treated on the stems with the chemical. Metcalf *et al.* (1957) found that R-6199 and related compounds were translocated from the stems to the leaves, where they were lethal to the two-spotted spider mite (*Tetranychus telarius* (L.)) and the greenhouse thrips (*Heliothrips haemorrhoidalis* (Bouché)). The chemistry, biological activity, and mode of action of these compounds have been discussed by Boon (1957).

In 1957, laboratory tests were conducted at Brownsville, Texas, to determine the effectiveness of R-6199 against 11 cotton pests and the residues found in cotton plants following foliage applications.

MATERIALS AND METHODS.—Cotton plants grown in 1-gallon cans were sprayed with technical R-6199 containing 75% of the oxalate salt dissolved in distilled water with 1% of the wetting agent Triton X-151. Each plant was placed on a turntable and sprayed with 2 or 4 ml. of a solution containing a known amount of the active ingredient.

In some tests, as soon as the spray deposits were dry, the treated plants were caged with insects and placed in a holding room where the temperature was maintained at 80° ± 2° F. In tests for residual effect, treated plants were kept in a greenhouse for various periods before the insects were introduced. In other tests, the lower and unfolded terminal leaves were infested separately. These terminal leaves were folded at the time of treatment. The temperatures in the greenhouse varied from 64° to 101° F. and the relative humidities varied from 26% to 91% during the testing period from August to October.

For the residue analyses all leaves from plants that had been sprayed at the rate of 5 mg. per plant were collected, weighed, and placed in a quart Mason jar containing 200 ml. of distilled water. The jar was rotated end over end at 60 r.p.m. in a tumbling machine for 30 minutes. The leaves were then rinsed with distilled water and air-dried. The leaves and washes were kept under refrigeration until chemical analyses were made.

Before analysis, the leaves were homogenized with 250 ml. of distilled water; the homogenate was allowed to stand for 24 hours with periodic shaking, and then filtered. The filtrates and aqueous washes were adjusted to pH 8.0 and tested for cholinesterase inhibition with human plasma as the source of the enzyme and acetylcholine chloride as the substrate. The amount of unhydrolyzed acetylcholine was determined by the colorimetric method of Hestrin (1949). The R-6199 concentrations were interpolated from the enzyme inhibition of the samples, using a standard curve constructed from measurements on a series of known concentrations of chemically pure R-6199 subjected to the same procedure. Aqueous washes and homogenates of untreated leaves were used as blanks. The amount of R-6199 residue found in the homogenate was considered the same as that found inside the leaves.

EFFECTS ON FOUR SPECIES OF LEPIDOPTEROUS LARVAE.—Second- and third-instar larvae of the salt-marsh caterpillar, the bollworm (*Heliothis zea* (Boddie)), the cabbage looper (*Trichoplusia ni* (Hbn.)), and the cotton leafworm (*Alabama argillacea* (Hbn.)) were caged on cotton plants, each of which had been sprayed with 4 ml. of a solution or emulsion containing 2 to 16 mg. of endrin, DDT, or R-6199. Ten to 20 larvae were introduced into each cage, and their mortalities were recorded 48 hours later. The data given in table 1 indicate that R-6199 was similar in effectiveness to endrin against all species except the bollworm, and that it was less effective than DDT or endrin against the bollworm.

Table 1.—Mortality of four species of lepidopterous larvae when exposed 48 hours to cotton plants treated with Chipman R-6199, endrin, and DDT.

Insect	Insecticide	Milligrams per Plant	Number of Insects	Per Cent Mortality
Salt-marsh caterpillar	Endrin	4	90	78.0
		8	121	84.0
	R-6199	4	61	82.0
		8	69	96.0
	Check	—	106	6.6
Bollworm	DDT	16	54	87.0
	Endrin	8	41	66.0
	R-6199	16	27	41.0
	Check	—	91	2.2
Cabbage looper	Endrin	8	128	82.0
	R-6199	8	87	70.0
	Check	—	127	8.7
Cotton leafworm	Endrin	2	62	100
	R-6199	2	80	96.0
	Check	—	113	5.3

RESIDUAL EFFECTS ON SPIDER MITES, APHIDS, THRIPS, MEALY BUGS, AND SCALE INSECTS.—In an experiment carried out in the greenhouse from August to October, two groups of six plants were treated on different dates with 5 mg. of R-6199 in 2 ml. of water per plant. Adults and nymphs of the two-spotted spider mite and the cotton aphid and nymphs of the thrips (*Hercothrips phaseoli* (Hood)) were introduced onto two treated and two untreated plants at 5- to 7-day intervals, about 50 of each species per plant.

The spider mites did not survive more than 24 hours when they were introduced on leaves of plants sprayed 1 to 7 days earlier. They were able to survive and reproduce on terminal leaves of plants infested 14 days after treatment. A few mites were able to survive for 48 hours on the lower leaves of plants sprayed 22 days prior to this introduction. When plants sprayed 48 days earlier were infested, no mites, including second-generation nymphs, could survive 7 days on lower leaves. Complete control of cotton aphids and thrips was obtained for 25 and 48 days, respectively, on lower leaves of treated plants. A shorter period of control was obtained on the terminal leaves. Infestations in all experiments were terminated on the 48th day after the initial treatment.

Crawlers of soft scale (*Coccus hesperidum* L.) failed to survive when they were introduced on the stems of plants sprayed 2 days earlier, but a few individuals survived on plants that had been sprayed 19 days earlier. No control of the mealy bug (*Phenacoccus solenopsis* Tinsl.) was obtained with a 1-day-old residue. The periods within which this chemical gave 100% control of these insects are given in table 2.

Untreated as well as treated plants used in the experiment were all naturally infested with the whitefly

Table 2.—Number of days Chipman R-6199 gave 100% control of some cotton pests when applied at 5 mg. per plant.

INSECT	LOWER LEAVES	TERMINAL LEAVES
Two-spotted spider mite	48	7
Cotton aphid	25	7
Thrips	48	20
Mealy bugs	0	0

(*Trialeurodes abutilones* (Hald.)). A few spider mites, (*Tetranychus cinnabarinus* (Bois.)) were found on the untreated but not on the treated plants throughout the test period.

RESIDUAL EFFECT ON PINK BOLLWORM AND BOLL WEEVIL ADULTS.—Cotton plants that had been sprayed with 5 mg. of R-6199 per plant were kept in the greenhouse until used in tests. At various intervals after the spraying, three plants comprising replicate were taken from the greenhouse to the holding room, where each plant was caged with 30 two-day-old boll weevil adults (*Anthonomus grandis* Boh.) or about 20 newly emerged pink bollworm moths (*Pectinophora gossypiella* (Saund.)). The tests were replicated three times for the pink bollworm and twice for the boll weevil. Net mortalities after 48 hours are given in table 3. These data indicate that residues older than 2 days were not very effective against adults of either insect. This drop in effectiveness corresponds to the rapid fall of R-6199 residues on leaf surfaces. (Table 6.)

R-6199, DDT, and a 1:3 mixture of these materials were tested on pink bollworm moths. One hour after treatment each plant was caged with 20 to 30 moths for 48 hours. Each dosage was replicated three times, each replicate consisting of one plant. Mortality data and median lethal dosages (LD-50's), which were estimated from visually fitted dosage-mortality curves, are given in table 4. The results indicate that the mixture was slightly more effective than DDT, based on DDT equivalent of the mixture calculated according to Wadley (1945). Since DDT is generally considered to have a different mode of action from organic-phosphorus insecticides, this higher effectiveness of the mixture indicates an additive action between DDT and R-6199. Similar results were also obtained on boll weevil adults when they were sprayed with the same mixture (unpublished data). The data in table 4 also indicate that the mixture at 16 mg. per plant was as effective as R-6199 at the same rate, or as DDT at 32 mg.

RESIDUES IN COTTON PLANTS.—Metcalf *et al.* (1957) found no evidence of metabolic changes of radioactive *O,O*-diethyl *S*-(2-diethylamino)-ethyl phosphorothioate (the free amine) or the oxalate (R-6199) to other toxicants when detected by paper chromatography or paper electrophoresis of chloroform extracts or juices of treated lemon or cotton leaves. However, in the presence of tap water (pH 7.5), the oxalate formed the free amine, which penetrated leaf cuticles and was readily translocated throughout the plant (Plant Protection Ltd. 1957).

In the study reported here, whether the residue was the free amine or the oxalate, or both, was not determined. According to C. L. Baldit (personal communication), there was no significant difference between the two materials in their inhibition of cholinesterase in human plasma when the solutions were adjusted to pH 8.0 before the addition of the enzyme. Therefore, in his opinion, the residue analysis, using the basified oxalate as standard, is valid. However, according to Metcalf *et al.* (1957) the oxalate was more potent than the free amine in inhibition of house fly cholinesterase.

In one series of tests the effect of time between the collection and washing of the leaves on the penetration of R-6199 was determined. Leaves taken from three plants were washed with water 1 hour after collection, and those from two plants were placed in plastic bags without washing and kept under refrigeration for a week before they were chemically analyzed. The residues found inside the leaves and on leaf surfaces (in wash) are given in table 5. These data show that the residue inside the leaves was higher when the leaves were washed a week after the collection than when they were washed after 1 hour. This difference indicates that detached leaves can absorb the chemical during storage and shipment.

In another series of tests the amounts of R-6199 that penetrated into leaves were determined. Leaves were collected from treated plants at 2-day intervals for 8 days. These tests were replicated three times, each replicate consisting of three plants. Leaves were also collected after 21 and 39 days from three plants each without replication, and the leaves were washed immediately. The results, given in table 6, show a sharp decrease of R-6199 residue in washes in the first 2 days but a gradual in-

Table 3.—Mortalities of boll weevil and pink bollworm adults in 48 hours on cotton plants sprayed with Chipman R-6199 at 5 mg. per plant.

INTERVAL BETWEEN APPLICATION AND TEST	BOLL WEEVIL		PINK BOLLWORM	
	Number of Insects	Per Cent Mortality	Number of Insects	Per Cent Mortality
1 hour	179	97.2	150	73.1
2 days	180	35.1	210	51.6
4 days	180	20.5	180	41.4
6 days	180	16.7	180	12.8

Table 4.—Mortalities of pink bollworm moths resulting from 48-hour exposure to cotton plants sprayed with DDT, Chipman R-6199, and a 1:3 mixture of the two materials.

DOSAGE (MG./PLANT)	NET PER CENT MORTALITY	LD-50 (MG./PLANT)	RELATIVE TOXICITY
DDT			
4	38	7.52	1.00
8	44		
16	63		
32	93		
R-6199			
2	19	3.20	2.35
4	74		
8	77		
16	90		
Mixture			
2	24	4.32	1.74
4	41		
8	82	5.92[a]	1.27[a]
16	91		

[a] Based on DDT equivalent of the mixture.

Table 5.—Effect of interval between collection and washing on the penetration of Chipman R-6199 into sprayed cotton leaves.

Interval Between		Fresh Weight of Leaves (Grams)	Residue (p.p.m.)		Per Cent Penetration
Treatment and Collection	Collection and Washing		In Wash	In Leaves	
1 hour	1 hour	58.1	24.0	6.0	20.0
	1 week	37.1	1.3	22.6	94.5
2 days	1 hour	36.9	13.0	16.4	56.5
	1 week	37.0	1.7	27.3	94.1
4 days	1 hour	35.0	6.5	22.0	77.1
	1 week	29.2	4.9	14.3	74.5

crease inside the leaves. Similar results were obtained by Metcalf *et al.* (1957) when cotton leaves were dipped into a solution containing radioactive R-6199. Table 6 also shows that the total residues remained at a stable level after the second day.

There was translocation of R-6199 from lower leaves to new leaves. The terminal leaves, which were not sprayed with the chemical, contained 14% and 29% of the total residues after 21 and 39 days, respectively. The amount of R-6199 in the wash from terminal leaves was much higher than that inside of the leaves. The presence of large translucent areas on the leaves indicated that R-6199 was washed out of the leaves during the 30 minutes' wash.

Table 6.—Residues in leaves from three cotton plants each treated with 5 mg. of Chipman R-6199.[a]

Interval Between Treatment and Collection	Fresh Weight of Leaves (Grams)	Residue (p.p.m.)			Per Cent Penetration
		In Wash	In Leaves	Total	
1 hour	48.8	45.3	13.5	58.8	23.0
2 days	36.9	14.1	21.3	35.4	60.0
4 days	29.2	8.8	17.2	26.0	66.0
6 days	35.5	2.7	23.8	26.5	90.0
8 days	35.2	2.3	26.5	28.8	92.0
21 days	69.4	2.8	13.3	16.1	82.6
Lower only	61.5	0.8	14.8	—	95.0
Terminal only	7.9	18.0	2.0	—	10.0
39 days	67.2	6.7	1.4	8.1	17.3
Lower only	56.6	5.3	1.5	—	22.1
Terminal only	10.6	14.0	0.9	—	6.0

[a] Both lower and terminal leaves unless otherwise indicated.

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Toxicological Action of DDT on Three Species of Mosquito Larvae[1]

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Abstract

In tests by radiometric methods the amount of DDT picked up and its relation to the mortality of fourth-instar larvae of *Anopheles quadrimaculatus* Say, *Aedes taeniorhynchus* (Wied.), and *Aedes aegypti* (L.) varied with the exposure time and the concentration. Larvae did not excrete DDT except when exposed to concentrations above the minimum LC-100. The toxic action of DDT on *quadrimaculatus* larvae differed from that on *aegypti*. Resistance to DDT in a strain of *taeniorhynchus* was not related to the uptake or the excretion. Live *quadrimaculatus* larvae absorbed three times as much DDT as dead larvae; however, in a 24-hour test period the surviving larvae had about the same dose as the nonsurvivors.

Studies on the toxicological aspects of DDT poisoning in mosquito larvae have been limited because the small quantities present in larvae could not be assayed by ordinary methods of chemical analysis. The availability of C^{14}-DDT and of radiometric methods of analysis made possible a study of the amount absorbed in a standard larval test method, its correlation with mortality, and the correlation of both these phenomena with exposure time and concentration.

In previous experiments (Schmidt & Weidhaas 1958) the mortality of fourth-instar larvae of *Anopheles quadrimaculatus* Say and *Aedes taeniorhynchus* (Wied.) was correlated with the amounts of three insecticides—DDT, dimethoate, and Co-Ral (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate, also known as Bayer 21/199)—found in the larvae after exposure for 24 hours. Plotting the dose absorbed in 24 hours against the concentration gave a straight-line relationship on either an arithmetic or a log-log scale, although the slopes varied with the species and insecticide. These and other data (Schmidt & Weidhaas 1959) show that the mortality of *quadrimaculatus* larvae was correlated with the dose of DDT absorbed in 24 hours even when the mortality varied with differences in test conditions such as the volume of suspension, number of larvae, and size of the container.

In the experiments described here, three species of mosquito larvae were exposed to suspensions of DDT to study the relationship between the concentration, the exposure time, the amount of DDT absorbed by the

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larvae, and the mortality. Experiments were also run to determine whether the larvae excreted or stored the DDT, whether there was a difference in the amounts of DDT found in larvae that had survived and those killed by the treatment, and whether larvae killed prior to exposure absorbed the same dose as did live larvae.

ABSORPTION AND MORTALITY.—Fourth-instar larvae of *quadrimaculatus*, *taeniorhynchus*, and *Aedes aegypti* (L.) obtained from laboratory colonies were used. Larvae of the *taeniorhynchus* colony were moderately resistant to DDT (about tenfold), whereas those from the other two colonies showed no resistance to this insecticide. The testing technique was similar to that described by Weidhaas & Gahan (1958). In each test 25 larvae were exposed to 250 ml. of a DDT suspension (an undetermined portion was in solution) made by adding an acetone solution of radioactive DDT (*p,p'*-DDT-4-C^{14}, having an activity of 2.1 μc./mg.) to distilled water. Tests were made in glass jars 3 inches in diameter.

The absorption of DDT was studied by exposing the larvae to three concentrations for periods ranging from 2 to 24 hours. All tests were run in triplicate. Mortality readings were made at the end of each exposure period. The larvae were then removed from the suspensions by screening, washed with 250 ml. of distilled water to remove any of the toxicant adhering to the integument, and ground with a tissue grinder. Before this procedure was established, a comparison was made between washing larvae with acetone and washing them with water; there was no difference in the radioactivity of larvae washed with water or acetone, indicating that washing with water gave complete removal of the external DDT. The ground larvae were plated on stainless steel planchets, and the radioactivity was assayed in a windowless gas-flow proportional counter. The counting data were corrected for self-absorption. The amount of DDT or its metabolite(s) present within the larvae was computed from the radioactivity. In this paper the absorbed dose and absorption refer to the entire amount of radioactivity, expressed as DDT, found within the larvae and include ingestion as well as absorption through the body wall.

Previous studies (Bowman *et al.* 1959, Weidhaas *et al.* 1959) have shown that loss of DDT by codistillation with water vapor influences the mortality of mosquito larvae. A comparison was therefore made of the dose absorbed by *quadrimaculatus* larvae exposed for periods ranging from 2 to 24 hours to 0.0035 p.p.m. of DDT in open jars and jars sealed with aluminum foil.

As shown in table 1, the dose absorbed increased with the concentration at a given exposure time and with the time at a given concentration for all species except with *taeniorhynchus* larvae between 19 and 24 hours. The rate of increase was similar for all three species and became progressively smaller as the exposure time lengthened.

When the concentrations at a given time differed, the increase in the amount absorbed per unit of concentration was different with each species, although with a given species it was constant. Weidhaas *et al.* (1959) showed a correlation between the distribution of DDT in test jars and larval mortality, which may account for the differences between species.

RELATION BETWEEN ABSORBED DOSE AND MORTALITY.—Because of the test design, mortality data did not cover the proper range for precise determination of the LC-50's and LD-50's or slopes of regression lines. Where sufficient

Table 1.—Mortality of three species of mosquito larvae and dose of DDT absorbed during exposures to various concentrations for various periods.

CONCENTRATION (P.P.M.)	HOURS OF EXPOSURE	PER CENT MORTALITY	DOSE ABSORBED (μG./LARVA $\times 10^{-4}$)
	Aedes aegypti		
0.001	2	0	3
	4	0	4
	6	0	6
	8	0	8
	19	0	14
	24	8	15
.003	2	0	8
	4	0	18
	6	0	21
	8	14	29
	19	81	35
	24	88	38
.01	2	0	20
	4	0	46
	6	31	57
	8	40	71
	19	100	97
	24	100	98
	Anopheles quadrimaculatus		
0.004	2	0	17
	4	0	24
	6	5	31
	8	3	36
	19	0	42
	24	5	53
.007	2	0	22
	4	0	31
	6	8	37
	8	20	45
	19	33	64
	24	41	65
.01	2	0	24
	4	0	38
	6	55	43
	8	64	47
	19	77	62
	24	80	67
	Aedes taeniorhynchus		
0.04	2	0	140
	4	4	290
	6	11	350
	8	3	410
	19	28	620
	24	46	560
.06	2	0	220
	4	0	320
	6	11	490
	8	11	530
	19	12	790
	24	63	720
.1	2	0	310
	4	7	410
	6	31	540
	8	29	650
	19	47	990
	24	41	920

mortality occurred, it correlated with concentration and dose absorbed at a given exposure time and with time and dose absorbed at a given concentration. Since mortality was increasing as the rate of absorption (μg./larva/hour) was decreasing, the dose absorbed was not the only factor controlling mortality. Furthermore, the total absorbed dose required to give a certain mortality varied with the concentration and time at which the dose was acquired. With *quadrimaculatus* an 8-hour exposure to 0.01 p.p.m. resulted in an absorbed dose of 0.0047 μg./larva and 64% mortality, whereas a 24-hour exposure to 0.004 p.p.m. resulted in an absorbed dose of 0.0053 μg./larva but only 5% mortality. Thus with this species a high concentration pressure over a short time gave greater mortality with a smaller absorbed dose than low concentration pressure over a long exposure time. With *aegypti* the results were just the opposite. When these larvae were exposed to 0.01 p.p.m. for 8 hours, the dose absorbed was 0.0071 μg./larva and the mortality 50%, whereas exposure for 24 hours to 0.003 p.p.m. resulted in an absorbed dose of 0.0038 μg./larva and 88% mortality. These results showed not only a species difference but also that the total dose absorbed cannot be related to mortality unless the two parameters of time and concentration are defined. Even then it is not the only factor controlling mortality. The mortality of *taeniorhynchus* larvae was inconsistent, owing to the heterogeneous nature of this resistant strain.

The relationship of the mortality to the dose absorbed differed with the species. With *aegypti* there was a much greater increase in mortality per unit increase in the dose absorbed than with *quadrimaculatus*, as shown by the slopes in figure 1. This difference may be due to the slightly larger absorbed dose of DDT found in *aegypti* (table 1) or a difference in the toxic action of DDT in the two species. Data for *taeniorhynchus* were erratic and too low for good correlation.

The results obtained with *taeniorhynchus* larvae, which were resistant to DDT, were compared with results obtained with a more susceptible strain in the earlier study (Schmidt & Weidhaas 1958). The increase in the dose absorbed with increasing concentration was similar in the two strains, although the concentrations to which the susceptible strain was subjected (0.004 to 0.01 p.p.m.) were only one-tenth as great as those used for the resistant strain. The resistance in the latter strain recorded by Davis *et al.* (1959) was therefore not related to the absorption of the chemical.

FACTORS DECREASING RATE OF ABSORPTION.—Some possible causes for the decrease in the rate of absorption as the exposure period increased were considered to be (1) mortality of the larvae, (2) decrease in the concentration of DDT in the suspension by codistillation or absorption by the larvae, (3) approach of a condition of equilibrium by which larvae either become saturated with DDT or degrade and/or excrete the DDT at a rate that increases with the internal concentration, and (4) cessation of feeding.

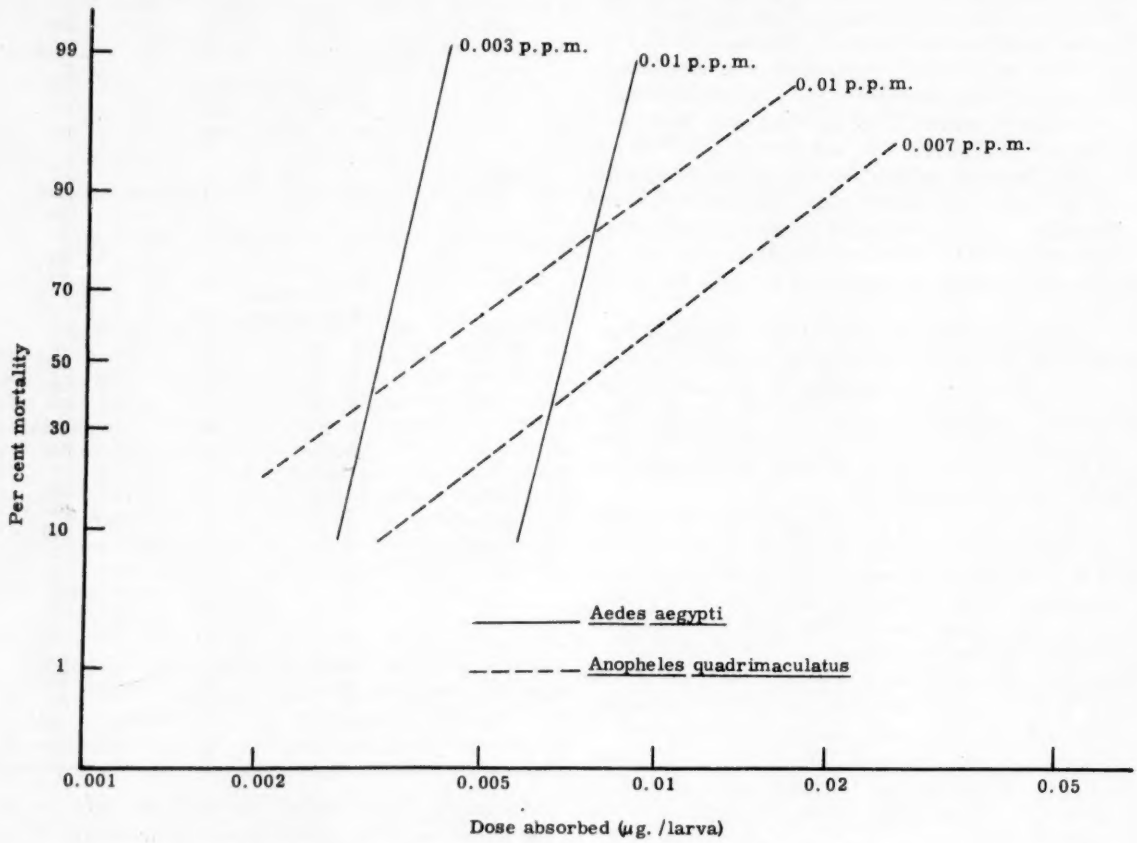

FIG. 1.—Relationship between per cent mortality and absorbed dose in mosquito larvae exposed to various concentrations of DDT for 2–24 hours.

In tests not shown in table 1, larvae of *quadrimaculatus* that survived a 24-hour exposure to 0.007 p.p.m. of DDT showed approximately the same absorbed dose (0.0056 μg./larva) as those that were killed during the exposure (0.0052 μg./larva), indicating that mortality during exposure was not the cause of the progressive decrease in the rate of absorption. These data also indicate that the difference in susceptibility was not caused by a difference in absorption, but rather by differences within the larvae such as metabolism and storage.

In order to observe the effect of the codistillation of DDT on the rate of absorption, a comparison was made of the doses absorbed by *quadrimaculatus* larvae exposed for various periods to 0.0035 p.p.m. of DDT in open and sealed jars. The absorbed doses (μg./larva$\times 10^{-4}$) were as follows:

	Total		*Rate per Hour*	
Hours	*Open Jars*	*Sealed Jars*	*Open Jars*	*Sealed Jars*
2	14	14	7	7
4	19	22	4.8	5.5
6	26	26	4.3	4.3
8	25	29	3.1	3.6
19	35	44	1.8	2.3
24	37	44	1.5	1.8

The absorbed dose was about the same in both sets of jars during the first 6 hours, but thereafter it was slightly higher in the sealed jars, indicating that codistillation did have some effect. This was not the only factor, since the rate of absorption decreased almost as fast in the sealed jars, but it had a significant effect on the mortality, which reached 5% in the open jars and 39% in the sealed jars after 24 hours. There was no mortality in either set of jars at 19 hours, by which time most of the decrease in the rate of absorption had occurred. This offers further proof that the decrease was not caused by larval mortality. Excretion of DDT or its metabolites was apparently not a factor in the decreasing rate of absorption.

To study the possibility of excretion of DDT, larvae of the three species were exposed to DDT over periods ranging from 1 to 5 hours. In each test jars were set up in sextuplicate. At the end of the exposure, mortality readings were made and the radioactivity of the larvae from three of the jars was determined. The larvae from the other three jars were transferred to clean water for the remainder of a 24-hour testing period. At that time the mortality was again recorded and the radioactivity determined.

Larvae of *quadrimaculatus* exposed to 0.01 p.p.m. and of *taeniorhynchus* exposed to 0.1 p.p.m. for 1 to 5 hours and then transferred to clean water apparently did not excrete an appreciable amount of the absorbed dose (table 2). These concentrations are below the minimum 24-hour LC-100. Larvae of *aegypti* exposed to 0.01 p.p.m. may have excreted a small amount, but this concentration was greater than the minimum 24-hour LC-100. Larvae of *quadrimaculatus* exposed to 0.04 p.p.m., well above the minimum LC-100, excreted 39% of the absorbed DDT when transferred to clean water. It appeared that the larvae did not excrete DDT absorbed in short exposures except when exposed to high concentrations. No data were obtained to study excretion in a continuous 24-hour exposure to normal test concentrations. The data in table 1 suggested that the dose absorbed did not influence the resistance in this strain of *taeniorhynchus*. Since these larvae did not excrete DDT at normal test concentrations and short exposure periods, the resistance in this strain is apparently a result of metabolism and/or innocuous storage within the larvae.

Table 2.—Mortality of mosquito larvae and dose of DDT absorbed at end of various exposure periods and after subsequent periods in clean water sufficient to complete a 24-hour test period.

		At End of Exposure		At End of 24 Hours	
Concentration (p.p.m.)	Hours of Exposure	Mortality[a] (%)	Dose Absorbed (μg./Larva $\times 10^{-4}$)	Mortality (%)	Dose Absorbed (μg./Larva $\times 10^{-4}$)
		Aedes aegypti			
0.01	1	0	21	5	15
	2	0	31	41	29
	3	0	37	47	33
	4	0	54	47	44
	5	20	58	52	51
		Anopheles quadrimaculatus			
0.01	1	0	15	5	22
	2	0	25	13	28
	3	0	31	28	32
	4	9	32	32	30
	5	25	45	53	40
.04	2	0	110	80	67
		Aedes taeniorhynchus			
0.1	1	0	200	27	250
	2	0	280	32	290
	3	0	360	35	300
	4	39	340	36	360
	5	75	390	49	390

[a] Includes moribund larvae.

When exposed for 6 hours to 0.007 p.p.m. of DDT, live larvae absorbed three times as much DDT as larvae killed with carbon dioxide (0.0033 vs. 0.0011 μg./larva). This difference could be due to the feeding action of live larvae as well as to the reduced rate of penetration through the body wall of dead larvae.

The full effect of the other possible causes for the decreasing rate of absorption has not been adequately studied.

Whether larvae obtain the absorbed dose of DDT from suspensions by absorption through the body wall or by feeding is not completely clear. Richards & Cutkomp (1946) showed a correlation between the presence of chitinous cuticle and sensitivity to DDT. They proposed a hypothesis that the chitinous cuticle facilitated the entry of DDT by selectively concentrating the compound by absorption phenomena. Work by Bowman *et al.* (1959) showing that DDT, which is hydrophobic, tends to concentrate at water interfaces, suggests a further mechanism for the concentrating of DDT at the body wall of larvae. The present writers (Schmidt & Weidhaas 1958 and unpublished data) found that the percentage of DDT removed from test dispersions by mosquito larvae was higher than that of four organophosphorus compounds—parathion, Co-Ral, dimethoate, and *O,O*-diethyl *O*-naphthalimidophosphorothioate (Bayer 22408). This finding also supports the view that DDT is readily absorbed via the body wall. However, the behavior of the larvae during the test period indicates that the feeding mechanism—*i.e.*, the circulation of the suspension by movement of the mouth parts—is important.

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BHC Translocation from Treated Soil and the Effect on Growth of Red Clover[1]

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ABSTRACT

Greenhouse-grown 5-month-old Kenland red clover from Brookston clay loam and Crosby silt loam treated with 0.1, 1.0, 10, and 100 p.p.m. BHC was analyzed for BHC content by the Schechter-Hornstein method. Averages of 0.08, 0.16, 0.91, 13.84 p.p.m. BHC and 0.10, 0.24, 2.07, 14.53 p.p.m. BHC for the respective soils and concentrations were found in the clover hay.

BHC dosages of 200 p.p.m. in Brookston soil and 100 p.p.m. in Crosby did not affect germination. Brookston soil required twice the BHC concentration present in Crosby to produce an identical effect on growth. Stunting, malformation, and mortality of young plants was proportionate to BHC dosage. Complete recovery from phytotoxic manifestations occurred in plants surviving beyond the ninth week.

Translocation of BHC in vegetable crops was demonstrated by bioassay (Starnes 1950) and by the same method, Haines (1956) detected BHC in the guttated liquid of corn plants. Lilly & Fahey (1956) determined by quantitative chemical analysis the BHC content of corn and legume seeds grown on high dosage soil treatments. Practical application of the BHC translocation phenomenon was noted early at the Ohio Agricultural Experiment Station by Weaver & Haynes (1955), who obtained a 95% control of the meadow spittlebug (*Philaenus leucophthalmus* (L.)) on test-plot red clover following band applications of 2 pounds gamma isomer per acre in May 1951.

Ware (1959) observed that while initial deposits of granulated applications of BHC on alfalfa were much lower than similar spray applications, the harvest residues were higher than those of sprays. This phenomenon was attributed to accumulation of BHC translocated via the root system from granules deposited on the soil. Since BHC fed to dairy cattle persisted in the milk for some time (Ware & Gilmore 1959), forage from treated soils or from fields in which BHC had accumulated as a result of repeated application of granulated or other formulations of BHC to crops might be the source of residues in food consumed by human beings. The relationship of soil concentration of BHC and soil type to the quantity of BHC translocated was accordingly investigated using greenhouse-grown red clover as the experimental crop.

METHODS.—Clover for chemical analysis was grown on Brookston soil, a dark clay loam, and on Crosby soil, a yellow-brown silt loam. For each soil 12 No. 10 cans containing 2.5 kg. (dry weight) each were prepared for each BHC concentration (0, 0.1, 1.0 10, and 100 p.p.m.) by allowing 5 ml. of acetone bearing the requisite quantity of BHC[3] to evaporate from the center soil surface of the can, after which the contents were mixed by hand in an enameled tray. The containers were then planted with innoculated red clover seed (Kenland variety) and arranged upon the benches of a steam-heated greenhouse cubicle. Standard fluorescent fixtures having two 85-watt tubes were each installed 18 inches above the containers 46 days after planting. At the appearance of the first trifoliate leaves the clover was thinned to 30 plants per can and watered with solutions of ammonium nitrate, monobasic sodium phosphate, and potassium chloride at the rate of 40 pounds each of N, P_2O_5, and K per 2 million-pound acre. Soil moisture was maintained between 20% and 40% of field capacity throughout the 5-month growing period, while temperatures ranged from 70° to 85° F.

At maturity, green weight was recorded for each three-can sample. The hay was then shade-dried for 3 days, weighed, and stored in pint Mason jars at 0° F.

Five-gram portions of each sample were extracted with methylene chloride for 2 hours in a large (50-×250-mm. body) Soxhlet apparatus fitted with a Friedrichs condenser. By analysis of a series of repeated extractions of previously extracted material, the recovery of BHC for the first extraction was calculated at 95%. BHC content of the extracts was determined by the Schechter-Hornstein method (1952), as modified by Wylegala *et al.* (1956), and by Ware (1959). Optical density of the developed color was read on a Bausch and Lomb *Spectronic "20,"* converted to p.p.m. against values obtained for standard methylene chloride solutions of BHC, and corrected for recovery percentage. Average readings for the control plantings, 0.565 for Brookston and 0.474 p.p.m. for Crosby, were subtracted from those for treated samples. Both field-grown clover and samples grown in a greenhouse room devoid of treatment gave readings of

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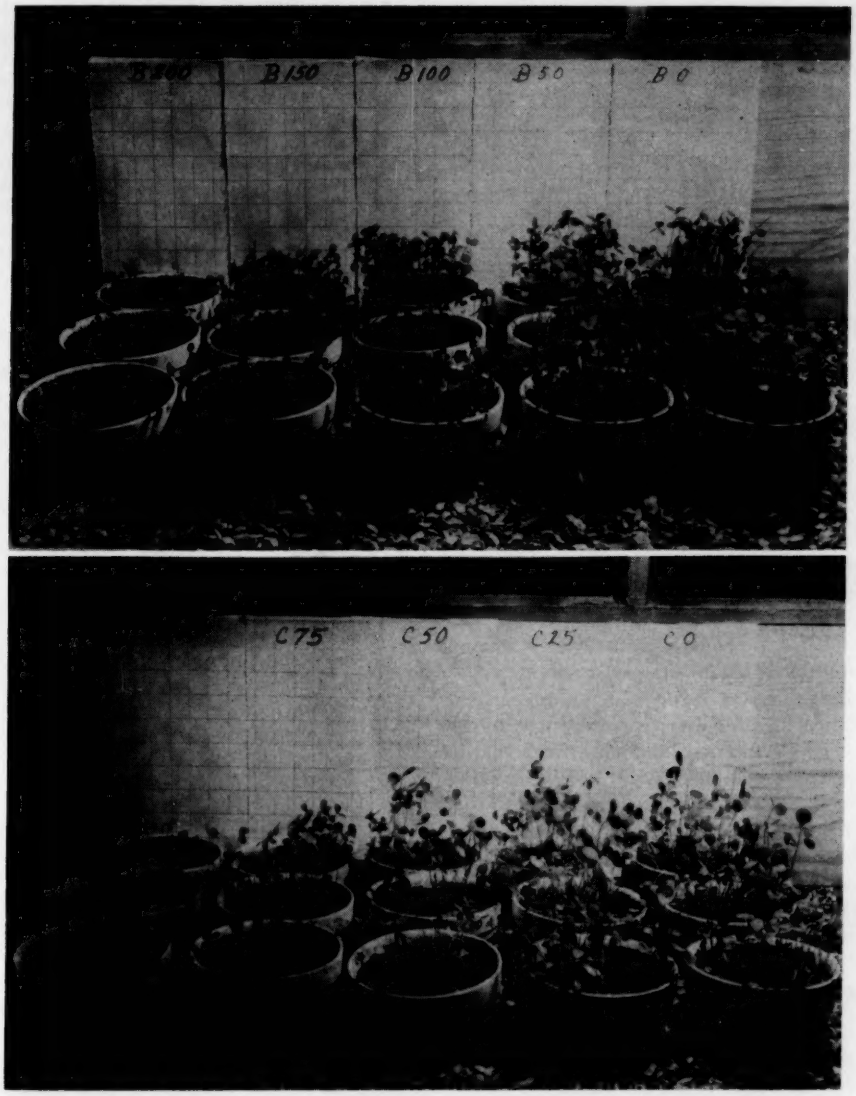
[2] Graduate Assistant and Assistant Professor, respectively.

[3] Technical BHC, 40% gamma isomer, supplied by United Cooperatives Inc., Alliance, Ohio



FIGS. 1 and 2.—Six-week-old red clover growing in 500 gm. soil samples treated with BHC. The initial letter of the soil (B—Brookston, C—Crosby) followed by the BHC concentration in p.p.m. designates each file.

only 0.02 p.p.m. Since analysis of the shellac can linings and the soil used for the control plantings did not reveal the source of the high control readings, the possibility of absorption of vapors transpired by BHC-bearing plants or volatilized from treated soils deserves consideration and investigation.

Germination and early growth were observed in plantings prepared by pressing 50 clover seeds into the firmed surfaces of finely mixed 500-gm. soil samples contained in earthenware cereal bowls. After the addition of 100 ml. of water, the bowls were covered with petri-dish lids and brown Kraft paper until germination counts had been made at 36 and 76 hours, using hypocotyl appearance as the criterion. Thereafter the treatment was essentially the same as for the analytical samples except that the plantings were not thinned. For this study the respective BHC concentrations in Brookston soil (0, 50, 100, 150, and 200 p.p.m.) were double those of Crosby (0, 25, 50, 75, and 100 p.p.m.), since observation of the early growth of samples for chemical analysis had indicated an estimated 2:1 (Brookston:Crosby) growth ratio per given dosage. At 9 weeks these plants were counted, cut, dried, and weighed.

RESULTS AND DISCUSSION.—No BHC concentration used had any observed effect on germination or survival prior to the appearance of the first leaf, although, at the higher concentrations, development was slowed and the first trifoliate leaves were malformed and chlorotic. A reduction of plant numbers proportionate to BHC dosage ensued at a gradually diminishing rate. Plants surviving beyond the ninth week, however, recovered completely from visible early phytotoxic effects and were equal to the controls in size and appearance by the end of the 16th week. Maximum differentiation of plants for the various treatment levels was attained during the sixth week (figures 1 and 2). At the higher dosages reduction of

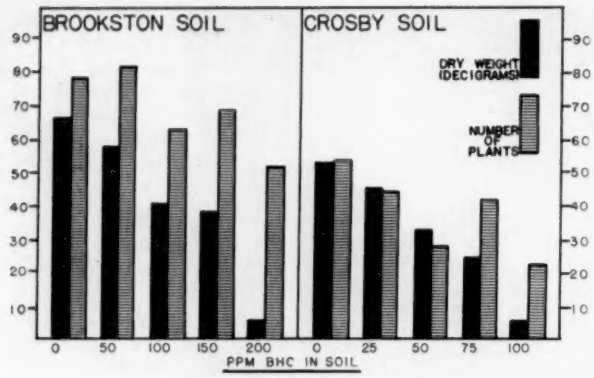


FIG. 3.—Ninth week dry weights and number of clover plants from BHC treated soils. Totals for each file of figures 1 and 2 are shown.

yield was more pronounced than the decrease in plant numbers (figure 3). Although absolute weights of clover produced on Crosby soil at one-half the dosage of Brookston were less, a 2:1 (Brookston:Crosby) dosage ratio per given effect was apparent when sample weights were expressed in per cent of the controls as shown in figure 4.

No marked reduction of yield was seen in plants grown to maturity at lower dosage levels (10 p.p.m. or less). The low yield shown in table 1 from Crosby soil at 100 p.p.m., the only higher concentration from which mature plants were harvested, was caused by a smaller number of plants, the survivors being as large and vigorous as those from other treatments.

The results of chemical analysis are shown in table 2. The severity of phytotoxic symptoms in young plants did not seem to indicate the amount of toxicant present in mature clover. Brookston soil at 200 p.p.m. and Crosby at 100 p.p.m. produced similar growth, while dissimilar effects at identical treatments of 100 p.p.m. resulted in

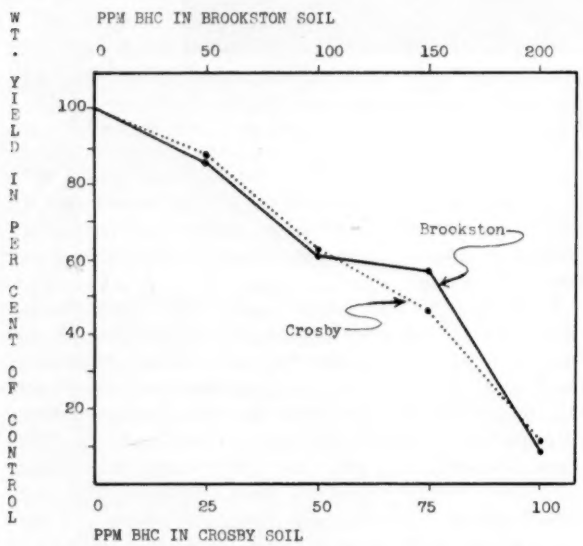


FIG. 4.—Ninth week dry weights of clover in terms of per cent of controls. Note that Brookston soil concentrations are double those of the Crosby counterparts.

Table 1.—Weights of clover hay from BHC-treated soils.[a]

BHC in Soil (p.p.m.)	Green Weight (Lb.)		Dry Weight (Lb.)	
	Brookston	Crosby	Brookston	Crosby
0.00	0.76	0.83	0.170	0.170
0.10	.59	.85	.130	.165
1.00	.60	.90	.130	.185
10.0	.66	.93	.150	.180
100.0	.79	.32	.155	.045

[a] Each weight is the total crop from nine cans.

Table 2.—Results of chemical analyses of clover grown on BHC-treated soils.

BHC in Soil	BHC in Clover Hay (p.p.m.)			
	Sample No.			
	1	2	3	Average
	Brookston			
0.10	0.08	0.14	0.03	0.08
1.00	.13	.28	.09	.16
10.0	.79	1.05	.88	.91
100.	13.75	14.59	13.19	13.84
	Crosby			
0.10	0.10	0.10	0.11	0.10
1.00	.23	.24	.26	.24
10.0	2.06	1.83	2.33	2.07
100.	13.78	13.43	16.38	14.53

hay samples which were almost equal in BHC content.

Adsorption on soil particles may conceivably make some of the BHC unavailable to the plant, or delay its availability. Brookston, which contains a higher proportion of fine clays and organic matter, at 200 p.p.m., and the larger particle Crosby soil at half that dosage were equal in their near-lethal effects. In a controlled trial of relatively inert sand-vermiculite (3:1) cultures, 50 p.p.m. BHC resulted in the death of all plants within 7 weeks.

Heavy BHC applications may exceed any adsorptive capacity of the soil, the excess providing nearly equal amounts of available toxicant in soils of different constituency. Soil factors may influence both quality and quantity of substances produced in plants. The control plantings of this experiment differed noticeably in that Brookston-grown clover was darker, had harder leaves and petioles, and had more and smaller leaves than the plants from Crosby soil. The lesser effect of BHC in Brookston soil may be caused by greater quantities of protective substances (Hopkins 1952), or to accumulations of waxes and oils which trap the toxicant because of greater partition coefficient in the plants. The increase in amounts of such materials in older plants may explain recovery from early phytotoxic effects.

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Vectors of Alfalfa and Bean Yellow Mosaic Viruses in Ladino White Clover[1]

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ABSTRACT

The clover aphid (*Anuraphis bakeri* (Cowen)), the yellow clover aphid (*Therioaphis trifolii* (Monell)), and the pea aphid (*Macrosiphum pisi* (Harr.)) transmitted alfalfa mosaic from infected to virus-free Ladino white clover plants, but only the clover aphid and pea aphid transmitted bean yellow mosaic. When fed on plants infected with both viruses, the clover aphid and the pea aphid transmitted each virus separately and indiscriminately. Neither virus was transmitted by the green peach aphid (*Myzus persicae* (Sulz.)), apple grain aphid (*Rhopalosiphum fitchii* (Sand.)), potato leafhopper (*Empoasca fabae* (Harr.)), clover leafhopper (*Aceratagallia sanguinolenta* (Prov.)), the strawberry spider mite, *Tetranychus atlanticus* (McG.) or the slug *Derocerus reticulatum* (L.).

Ladino white clover (*Trifolium repens* L.) is an important forage legume in many parts of the United States. Several viruses in this host are widespread and have been described (Houston & Oswald 1953, Kreitlow & Price 1949). It has been shown that virus infection can drastically reduce the yield, flowering and seed production of this clover (Kreitlow *et al.* 1957, Kreitlow & Hunt 1958), but there is little information concerning the spread by insects of nonpersistent viruses occurring in white clover. The pea aphid (*Macrosiphum pisi* (Harr.)) has been reported as a vector of alfalfa mosaic virus (AMV) and bean yellow mosaic virus (BYMV) (Swenson 1952, 1954). Swenson (1957) suggested that this aphid carried BYMV from clovers and alfalfa to other hosts. Clovers mentioned as susceptible to BYMV include white sweetclover (*Melilotus alba* Desr.), yellow sweetclover (*M. officinalis* (L.) Lam.), alsike clover (*T. hybridum* L.), crimson clover (*T. incarnatum* L.) and red clover (*T. pratense* L.). However, Swenson (1954) reported that the pea aphid was unable to transmit BYMV to Ladino white clover.

During the period 1956 to 1958 a study was made to determine which vectors can transmit AMV and BYMV from infected to virus-free plants of Ladino white clover.

MATERIALS AND METHODS.—The tests were conducted in winter and early spring months in an insectary and in regularly fumigated, screened greenhouses. A clone of Ladino white clover used in these studies had been previously selected for susceptibility to AMV and BYMV. A healthy plant of the clone, indexed for freedom from virus infection on Bountiful bean seedlings, was clonally divided into two parts. One part was clonally propagated and retained as virus-free. The other was clonally multiplied and three plants were inoculated—one with a strain of AMV, one with a strain of severe BYMV, and the third with a mixture of both viruses. Each of the lines was clonally increased to obtain sufficient plants for the tests.

Seven species of insects were tested as possible vectors—the pea aphid, the clover aphid (*Anuraphis bakeri* (Cowen)), yellow clover aphid (*Therioaphis trifolii* (Monell)), the green peach aphid (*Myzus persicae* (Sulz.)), the apple grain aphid (*Rhopalosiphum fitchii* (Sand.)), the potato leafhopper (*Empoasca fabae* (Harr.)), and the clover leafhopper (*Aceratagallia sanguinolenta* (Prov.)). A slug, *Derocerus reticulatum* (Muller), and the strawberry spider mite (*Tetranychus atlanticus* (McG.)) were also tested. Some suspected vectors were reared on plants of cabbage and wheat that were not known hosts for these viruses, but most of them were cultured on seedling legumes and cirus-free Ladino white clover.

Suspected vectors were starved for 1 to 3 hours. Unless indicated otherwise, they were confined for 1 hour on infected Ladino white clover plants or detached leaves before being transferred to virus-free plants. After 18 to 24 hours the plants were briefly immersed in a nicotine sulfate solution and incubated for at least 21 days at 65° to 70° F. Following incubation, each plant was indexed for virus infection by rubbing a gauze pad dipped in some of its juice on leaves of at least two Bountiful bean seedlings growing at 75° to 80° F. Transmission of AMV was indicated by development of characteristic reddish-brown local lesions on inoculated bean leaves within 48 to 72 hours, and of BYMV by development of severe stunting and mottling in newly emerged trifoliolate leaves within 7 to 12 days.

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[2] Entomology Research Division and Crops Research Division, respectively, Beltsville, Md., Agricultural Research Service, U. S. Department of Agriculture.

Table 1.—Virus transmission by three aphid species fed on AMV or BYMV-infected Ladino white clover plants growing in a greenhouse.

Aphid Species	Virus	Number of Aphids per Plant	Number of Test Plants	Number of Positive Transmissions
Clover aphid	BYMV	4	4	0
		10	25	5
		25	8	1
	AMV	4	4	0
		10	4	1
		25	8	1
Yellow clover aphid	BYMV	1	4	0
		2	3	0
		25	4	0
	AMV	1	4	0
		2	3	1
		25	4	0
Pea aphid	BYMV	10	20	0
	AMV	10	20	5

Table 2.—Virus transmission by three aphid species fed on greenhouse-grown Ladino white clover plants simultaneously infected with a mixture of AMV and BYMV.

Aphid Species	Number of Aphids per Plant	Number of Test Plants	Number of Positive Transmissions: Bean Yellow Mosaic	Alfalfa Mosaic	Both
Clover aphid	4	3	0	0	0
	10	20	1	2	0
	10	4	1	1	0
	5[a]	4	1	0	0
	—[b]	10	3	0	0
Yellow clover aphid	1	5	0	0	0
	2	3	0	0	0
	25	4	0	0	0
Pea aphid	10	24	2	2	0
	10	4	1	0	0
Total		81	9	5	0

[a] Infective feeding 15 minutes, transfer feeding 15 minutes.
[b] Aphids were allowed free transfer from infected to virus-free test plants. Number and length of feeding period could not be controlled.

To determine the kinds and numbers of insects that might be vectors occurring on plants of Ladino white clover, three fields in central Maryland were sampled at intervals during the growing season of 1957.

RESULTS.—Six of the nine suspected vectors failed to transmit either AMV or BYMV. These six species and number of transmission attempts were as follows: apple grain aphid 62, green peach aphid 22, potato leafhopper 62, clover leafhopper 9, strawberry spider mite 33, and slug 18.

The clover aphid, yellow clover aphid, and pea aphid transmitted AMV but only the clover aphid transmitted BYMV (table 1). Because of the low rate of transmission obtained, it is difficult to draw conclusions concerning the optimum number of aphids per plant needed to assure virus transmission.

When the aphid vectors were fed on plants infected with a mixture of AMV and BYMV, each virus was transmitted separately to virus-free plants by the clover aphid and pea aphid, but the yellow clover aphid failed to transmit either virus (table 2). In 81 test plants the 14 successful transmissions were of one or the other virus but never the mixture contained in the source plants.

Table 3 compares the numbers of different kinds of insects collected in three fields of clover in 1957. Aphids were not abundant in the fields sampled and were present only in spring and fall. Leafhoppers were collected during most of the season and exceeded aphids in number and in kinds of species. In addition to the three species of leafhoppers recorded, 14 others were collected as well as grasshoppers and many other insects.

DISCUSSION.—The clover aphid, which transmitted AMV and BYMV, and the yellow clover aphid, which transmitted only AMV in one test, are previously unreported vectors of these viruses. AMV was transmitted by the yellow clover aphid from AMV-infected plants in 1 out of 11 attempts, but not from a mixture on AMV and BYMV in 12 attempts. More extensive tests are needed to establish whether this aphid is a vector of AMV in Ladino white clover. The pea aphid, previously reported (Swenson 1952, 1954) as a vector of AMV and BYMV, transmitted AMV.

The pea aphid failed to transmit virus from BYMV-infected plants, but did transmit this virus in 3 out of 28 attempts when fed on plants infected with a mixture of AMV and BYMV. These data suggest that BYMV is more readily transmitted by aphids from white clover plants infected with a mixture of AMV and BYMV. Since genetically identical plants were used throughout the study, interaction between viruses, plant juice, and insects should not have been an appreciable factor. Some of the differences between aphid transmission of BYMV in this study and those reported by other workers may be due to differences in strains of virus used. Some strains of BYMV are apparently incapable of infecting white clover.

It has been shown that insects with similar feeding habits transmit some viruses but not others (Bawden 1950). When different species of aphids are fed on plants

Table 3.—Insect population in three fields of Ladino white clover in central Maryland sampled five times in 1957.

Insect	No. per 100 Sweeps[a]: June 13	July 8	July 30	Aug. 27	Oct. 16
Aphids:					
Pea aphid	4.4	0	0	0	7.0
Yellow clover aphid	0.1	0	0	0	0
Clover aphid	0	0	0	0	0
Cowpea aphid	0	0	0	0	0.7
Leafhoppers:					
Potato leafhopper	0.4	9.6	1.8	0	2.0
Clover leafhopper	11.7	19.5	39.8	30.2	6.7
Agallia constricta Van D.	8.3	479.6	447.6	84.7	0
Grasshoppers:					
Acrididae	23.1	12.1	1.8	0.5	0.2
Locustidae	2.7	4.7	0.9	0.2	0

[a] Based on samples of 200 to 500 sweeps in each field.

infected with virus mixtures, one species transmits one virus to healthy plants and another species a different one. In the present study the clover aphid and pea aphid fed on plants infected with a mixture of AMV and BYMV transmitted each virus separately to virus-free plants. However, there was no relationship between aphid species and the virus transmitted. Each virus was transmitted with equal facility by the clover aphid and pea aphid. Why one or the other virus in a mixture was separately and indiscriminately transmitted by the two species of aphids is difficult to explain and seems to have no precedent in the literature. Further studies on this problem are in progress.

It is not at all surprising that the leafhoppers, the mite, and the slug failed to transmit AMV and BYMV, since these species have not been reported as transmitting these viruses.

The only aphid species found in the field that was not tested in this study was the cowpea aphid (*Aphis medicaginis* Koch.), which has been reported as a vector of AMV (Swenson 1952) and BYMV (Corbett 1958). The clover aphid was not collected in the fields sampled, although it was easily reared on Ladino white clover in the greenhouse and was collected on red clover growing in the vincinity of the white clover fields that were sampled.

Since at least three species of aphids have been shown experimentally to transmit one or both of these viruses in Ladino white clover, they probably play some role in spread of the viruses in the field. Since neither virus is seed-borne (Kreitlow & Hunt 1958), and since aphids appeared in the field in the spring and fall, perhaps the role of the aphids is that of initiating infection in a newly established stand. Rapid increase in infection with age of clover stand suggests that viruses can be spread by mowing, by grazing, or by insect vectors not yet demonstrated.

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A Sampling Technique for Estimating Populations of Small Arthropods in Soil and Vegetation[1]

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ABSTRACT

Apparatus and techniques were devised for estimating populations of arthropods in samples of soil and vegetation. The specimens are extracted from the media by agitating in water, and aliquots are withdrawn during agitation. For the recovery of soil-inhabiting species, the aliquots are allowed to settle and the clear liquid is run through a series of sieves attached to a vacuum pump. The specimens are concentrated in nylon sieves and counts are made on plastic counting disks. For insects on vegetation, aliquots taken in the same manner are run through Büchner funnels equipped with nylon screens, and the counts are made directly on the screens. Counts made with these techniques indicated recoveries having a mean standard error of about 10%. It is believed that this method may be adapted to almost any small arthropod where the specimens must be extracted from media such as soil and vegetation, and where the numbers are so great that aliquots must be taken to expedite counting.

When biological, ecological, and control studies of small arthropods are undertaken, considerable time and effort must be spent in devising apparatus and techniques. To estimate populations of these arthropods it is often necessary to extract them from media such as soil and vegetation. When large numbers are present, aliquots must be taken in order to save time in counting. This paper describes apparatus and techniques devised for estimating populations of eggs and active stages of the brown wheat mite (*Petrobia latens* (Müll.)) in soil, and of active stages of mites and other small arthropods in the foliage of small grains and sorghums.

POPULATIONS OF BROWN WHEAT MITE IN SOIL.—The brown wheat mite lays two types of eggs. In the Great Plains the active eggs are deposited and hatch in the fall, winter, and spring, and the diapause eggs are deposited in the spring and hatch in the fall. Both types of eggs, as

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well as recently hatched larvae and quiescent stages of the mite, are found on the dry basal leaves of host plants, under clods and debris, and within soil cracks. A method of estimating these populations in samples of soil and debris is described below.

Soil Sampling.—Samples consisting of clumps of small-grain stubble, surface debris, and underlying soil are taken with the device shown in figure 1. This sampler consists of a metal cylinder 5 inches in diameter and 5 inches deep attached to a handle of 1-inch pipe by two ¾-inch angle irons. The bottom of the cylinder has a saw-tooth edge. The sampler is placed over the clump and pushed into the ground with a twisting motion until the rim of the cylinder is even with the soil surface. After the core has been taken, it is placed in a paper bag which is then labeled. If the soil is dry or powdery, the core may be retained in the sampler until it is dropped into the bag by covering the end of the tube with the hand or a metal plate. When the soil is wet, plastic bags or waxed cartons may be used. The containers of soil are brought to the laboratory and held in a refrigerator at approximately 50° F. until time is available to extract the eggs and other soil-inhabiting stages of the mite.

Washing Unit.—The specimens to be counted are removed from the sample by agitating in water, as was done by Jones & Prendergast (1937), with an apparatus consisting of a washing unit and a screening unit. The unit (fig. 2) consists of an 860 r.p.m. electric motor which operates an offset drive wheel (*a*) attached by a connecting rod to an agitator arm (*b*). This arm makes 200 complete strokes per minute. Embedded in a keyway in the agitator is a length of bicycle chain that meshes with a 3-inch sprocket wheel attached by means of a bicycle front axle to an agitator (*c*) inserted in a 1-gallon round-type churn jar (*d*). The vertical position of the agitator is governed by raising or lowering the jar with two cranks that operate an elevator platform (*e*).

The sprocket wheel axle turns in a bronze bushing, and the lower end is attached to the male section of a ½-inch socket wrench. The agitator consists of eight $\frac{3}{16}$-inch metal tines flattened on the ends to a width of ½-inch, and welded to a 3-inch flange into which the female section of the socket wrench is attached. The agitator is fastened to the axle by snapping the male and female sections together. It is important that the female section is the type with a dimple on all four surfaces of the depression to accommodate the spring-loaded ball bearing mounted in the male section. This holds the agitator firmly in place regardless of how it is lined up with relation to the ball.

The soil core is emptied into a standard 10-inch tractor funnel placed in the neck of a churn jar. To prevent stubble and other large debris from entering the jar, a ½-inch mesh hardware-cloth screen is soldered over the 3-inch hole at the base of the funnel. By means of a coarse spray of water, the soil and small debris are washed through the screen. The water level is then brought up to the neck of the jar, the agitator inserted in the liquid, and the jar placed on the elevator platform directly beneath one of the agitator connections. When four jars of samples are in position on the platform, the sockets are snapped together and the platform is raised until the ends of the tines just clear the bottoms of the jars. An interval timer is then set, the motor switched on, and the samples are agitated for approximately 4 minutes. In the meantime a 1-pint settling jar (*f*) is placed on the bench directly beneath the outlet of each churn jar.

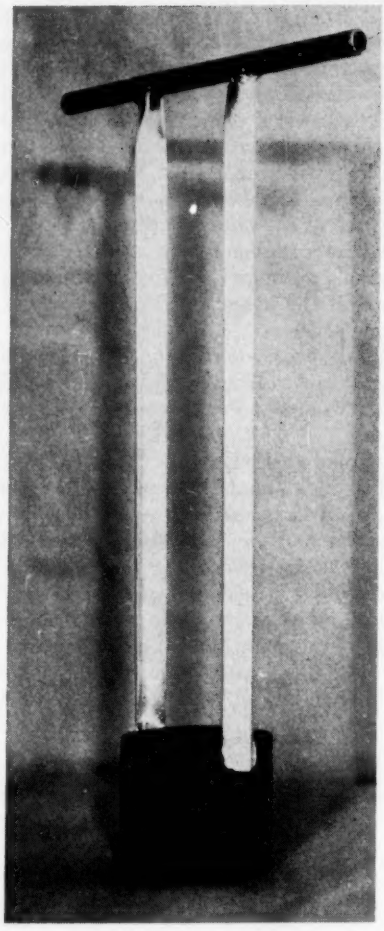

FIG. 1.—Sampling tube for collecting cores of soil and debris to estimate populations of soil-inhabiting stages of brown wheat mite.

While the samples are being agitated, an aliquot of liquid is removed from the churn jar by releasing the pinch clamp so that the liquid will flow through the outlet tube. Approximately ½ pint is first withdrawn and discarded, in order to eliminate the unagitated portion that is contained in the outlet tube. A 1-pint aliquot is then usually withdrawn, but when high populations are involved, it may be reduced to one-quarter pint or less. When active stages of the mite are present in the sample, a screw cap containing a paper clip soldered to the inside, is screwed on the settling jar. A piece of blotting paper inserted under the clip is saturated with carbon tetrachloride before the cap is attached. These aliquots are allowed to settle for 24 hours, or until the top liquid is relatively clear.

As soon as the last aliquot has been withdrawn, the motor is turned off and the elevator platform lowered. The agitators are unsnapped and the churn jars containing the muddy liquid removed to the bench. The agitators are then lifted from the jars and washed. The jars are quickly cleaned by inverting them over a sprinkler

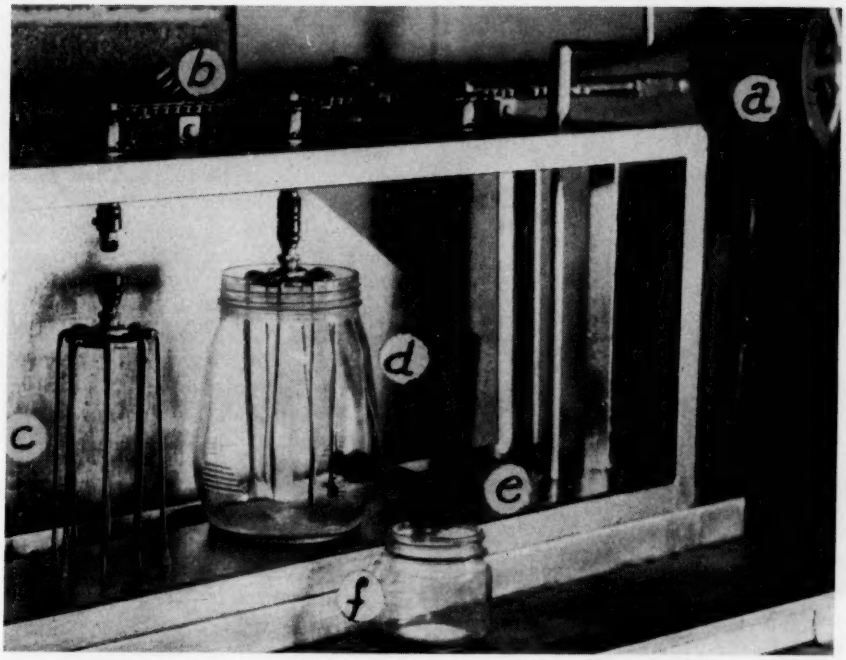

FIG. 2.—Washing unit for extracting specimens from soil samples and removing aliquots for screening: *a*, offset drive wheel; *b*, agitator arm; *c*, agitator; *d*, churn jar; *e*, elevator platform; *f*, settling jar.

nozzle mounted beneath a ¼-inch mesh screen rack fitted into a tractor funnel soldered to the bottom of the galvanized-iron sink. The spray of water washes the mud and debris into the funnel and down through a plastic tube which leads to a hole in the wall of the building.

Screening Unit.—To recover the eggs and other soil stages of the mite that are floating on the surface, the clear liquid in each settling jar is passed through a series of sieves (fig. 3) connected by means of a funnel base to a vacuum pump located under the bench. The pump is turned on and the liquid is poured into the top sieve (*a*) with a revolving motion to flush the entire rim of the jar and dislodge any specimens that may have adhered when the water level was lowered by evaporation during settling. (When the liquid has been removed, the jar is placed over the sprinkler nozzle mounted in the sink, and the sediment flushed down the drain.) The top sieve is sprayed for 10 seconds with water from a spray nozzle consisting of an air gun (*b*) attached to a 65.2 Sprayjet tip (*c*). This sieve is then removed and the middle one (*d*) sprayed for 40 seconds. If the water level in the middle sieve rises, because of plugging of the nylon screen in the bottom sieve, this screen is sprayed from below with water from a nozzle mounted in the funnel base (*f*) and operated by a foot control. This spraying clears a small area in the screen so that suction will be resumed. When the middle sieve is removed, the water spray is swept across the nylon screen in the bottom sieve (*e*). After the specimens from the settling jar have been concentrated in the nylon sieve, it is removed from the base, which is attached by means of pressure tubing to a 5-gallon reservoir bottle connected to a vacuum pump. The nylon sieves are then placed in a refrigerator at 50° F. until the contents are dry.

The screening units are constructed of standard 4-inch soil drying cans. The top sieve (fig. 4), which removes the large debris, has a funnel lip (*a*) soldered to the upper rim of the soil can (*b*) from which the bottom has been removed. A collar (*c*) consisting of the lid of the can with

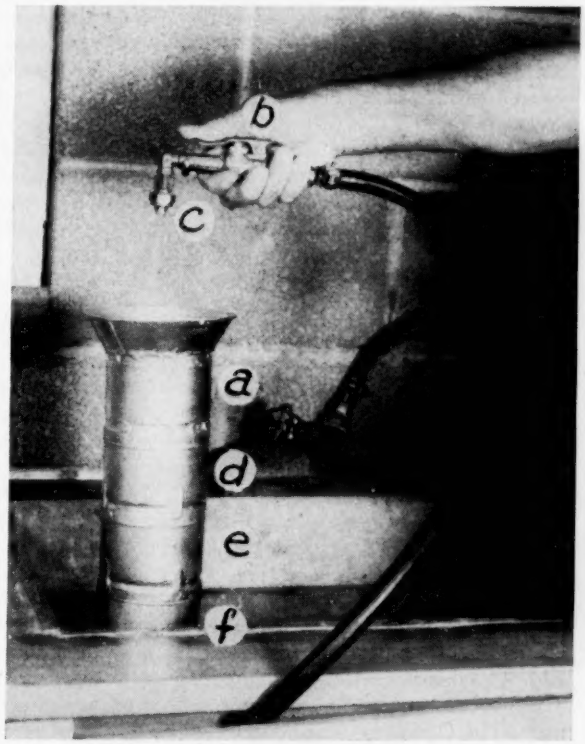

FIG. 3.—Screening unit for concentrating specimens in nylon sieve: *a*, 16-mesh sieve; *b*, air gun; *c*, spray tip; *d*, 60-×80-mesh sieve; *e*, 135-mesh nylon sieve; *f*, funnel base leading to reservoir bottle and vacuum pump.

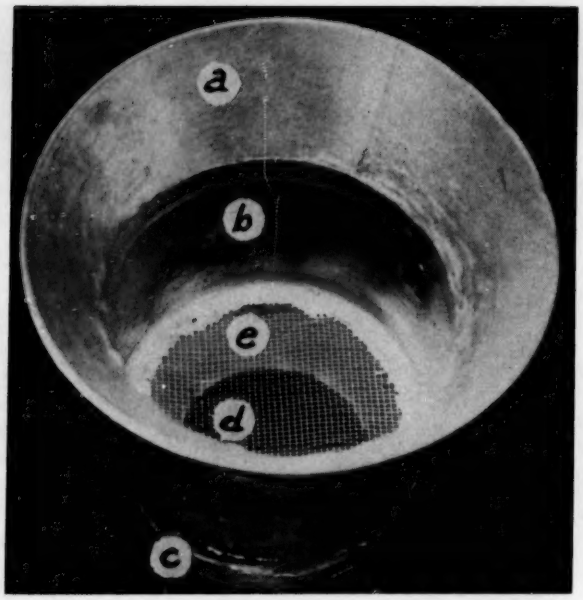

FIG. 4.—Top sieve of screening unit for removing trash and large debris: *a*, funnel lip; *b*, soil drying can; *c*, collar; *d*, 16-mesh screen; *e*, inner funnel.

the central area removed, is soldered to the lower rim and serves as an attachment when the sieves are joined. The bottom of the sieve is covered with 16-mesh metal screen (*d*), and an inner funnel (*e*) is soldered to the base to prevent liquid from leaking through the crack when the sieves are connected during screening. The middle sieve, which removes the small particles of sand and vegetative matter, is similar to the top one except that the base is covered with 60-×80-mesh metal screen and it does not have a funnel lip or an inner funnel. The bottom sieve (fig. 5) has the base of the soil can (*b*) covered with 135-mesh nylon screen (*d*). Four metal bridges (*f*) soldered to the collar (*c*) and the base of the sieve, make a strong connection between the two parts. The crack between the two is filled with liquid solder.

Other types of screening units may be made, and a fine wire-cloth screen may be substituted for nylon; however, the one described seems suitable for the purpose. Numerous tests have indicated that all the eggs of the brown wheat mite pass through the 60-×80-mesh screen, but none through the nylon screen.

Counting Unit.—The counting of specimens extracted from soil samples is done with the equipment shown in figure 6. After the contents of the nylon sieve (*a*) have become dry in the refrigerator, they are brushed into a metal ointment tin (*b*) through a funnel device (*c*) for later examination and counting. The counts are made under a binocular microscope having a magnification of about 14 diameters. The sample is emptied upon a plastic counting disk and spread evenly over the surface with a coarse pocket comb. This disk (*d*) is 5 inches in diameter and consists of 20 green and 20 white strips $\frac{1}{2}$-inch wide. Counts are made on either set of strips, or on both if the entire sample is to be examined. The count on half the area can be multiplied by 2 with little loss in accuracy. Collections to be held for future study are transferred from the counting disks to ointment tins by means of a chute (*e*) and stored in the freezing unit of the refrigerator.

Adequacy of Extraction Apparatus.—A large number of counts of active and diapause eggs, as well as newly-hatched larvae of the brown wheat mite, have been made from samples subjected to this washing and screening technique during the last 7 years. It was found that while the sample was being agitated the distribution of eggs in the liquid was so nearly uniform that the mean percentage of total eggs collected in the 1-pint aliquot from each of six churn jars (13.3 ± 1.3) had a standard error of less than 10%. Counts taken at other times indicated mean recoveries that ranged between 12.0% and 14.6% of the total population. The distribution of newly hatched larvae was equally uniform. When all such counts were averaged, it was determined that the populations of specimens within the entire sample could be closely estimated by multiplying the number in a 1-pint aliquot by the factor 7.94.

A number of soil cores were washed and screened three times to determine the percentage of diapause eggs recovered in each run. The percentages were as follows: first run 94, second run 4.8, and third run 1.2. Because of the high percentage of the eggs recovered during the first extraction, it is evident that little additional accuracy would be gained by subsequent runs.

POPULATIONS OF OTHER ARTHROPODS ON VEGETATION.—By a slight modification the washing unit may be used for recovering insects and mites from samples of vegetation, such as the corn leaf aphid (*Rhopalosiphum maidis* (Fitch)) from the whorls and heads of sorghum, eggs of the corn earworm (*Heliothis zea* (Boddie)) from

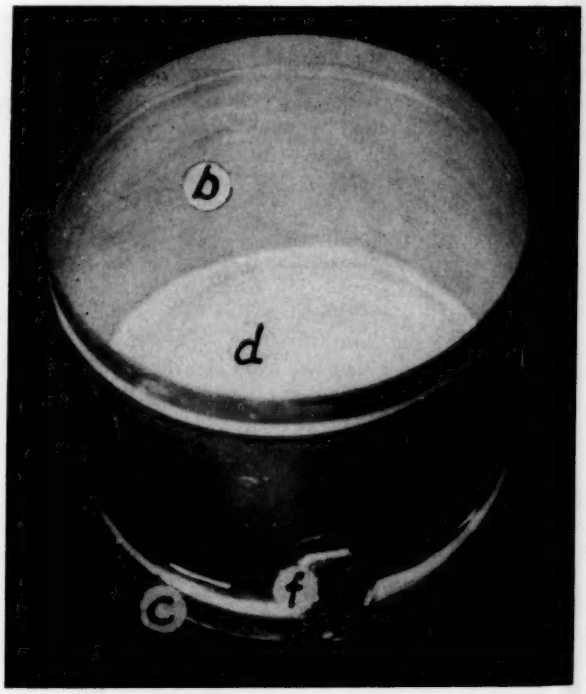

FIG. 5—Bottom sieve in screening unit on which specimens are confined: *b*, soil drying can; *c*, collar; *d*, 135-mesh nylon screen; *f*, metal bridge.

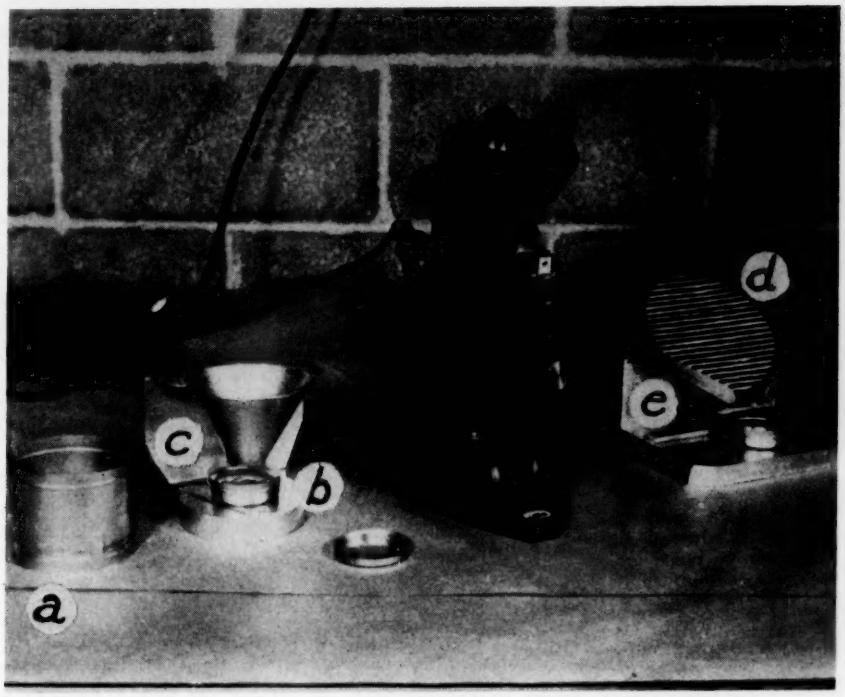

FIG. 6.—Counting unit: *a*, nylon sieve; *b*, ointment tin; *c*, transfer funnel; *d*, counting disk; *e*, transfer chute.

corn silks, and the date mite (*Oligonychus pratensis* (Banks)) from the crowns of small-grain plants. It is believed that this method may be adapted to almost any small arthropod where the specimens must be extracted from a medium such as soil or vegetation, and where the numbers are so great that aliquots must be taken to expedite counting.

For extracting arthropods from vegetation it is only necessary to substitute the agitator shown in figure 7 for the one with metal tines. This is a cylindrical basket made

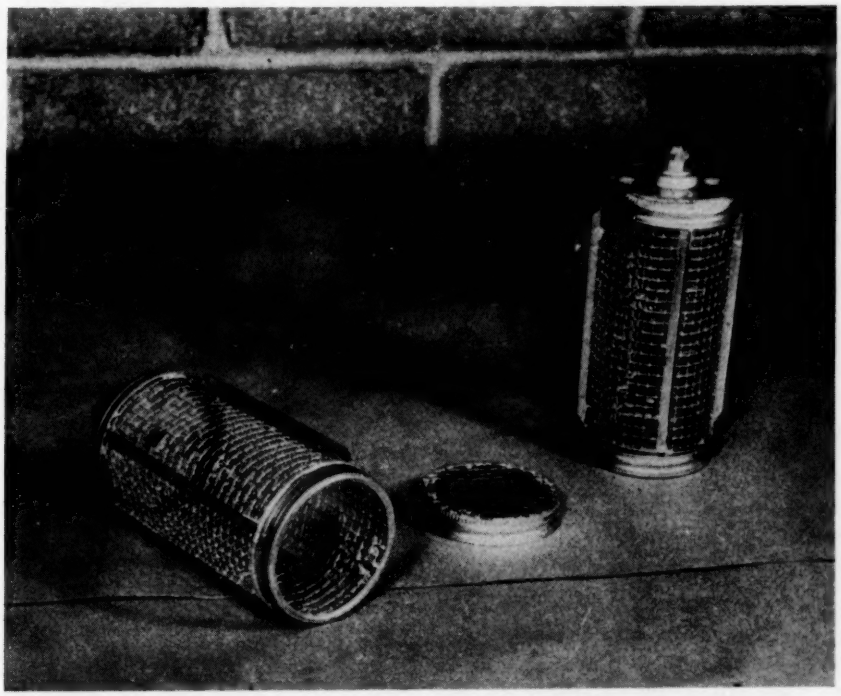

FIG. 7.—Agitator for extracting arthropods from vegetation.

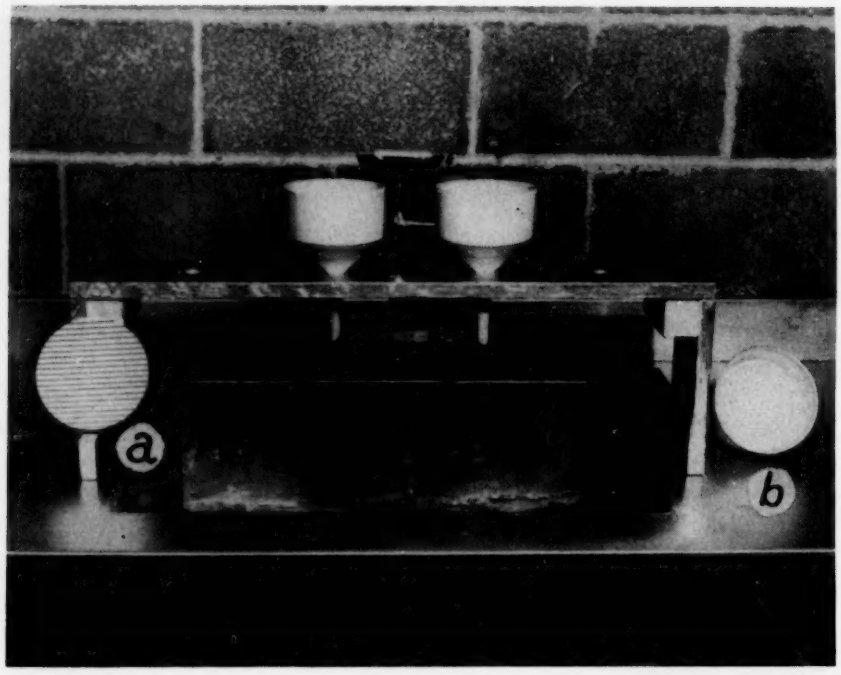

FIG. 8.—Screening apparatus for recovering arthropods from vegetation: *a*, nylon screen; *b*, Buchner funnel.

of ¼-inch mesh hardware cloth, the upper end of which is fastened to a 3-inch flange and the bottom to a fruit-jar ring that has been made slightly smaller so that another ring that forms the cap can be screwed over it. The cap is also covered with similar hardware cloth. At four equidistant points on the surface of the cylinder, ⅜-inch angle pieces are soldered the full length to serve as agitation fins.

A rack (fig. 8) of Büchner funnels is also necessary for recovering the insects extracted, together with a number of white nylon screens cut to fit the perforated filtering surface of the funnel. These screens are marked off in parallel lines just far enough apart to be included in the field of the wide-angle binocular microscope. For light-colored insects the nylon may be dyed to furnish a contrasting background.

The method of extraction is quite different from that of recovering eggs and other stages of arthropods from the soil. The sample is placed in the agitator basket, which is then inserted in the churn jar filled to the neck with water containing a small amount of detergent. The motor is then switched on and the sample agitated for about 5 minutes. While the samples are still being agitated, an aliquot of liquid is removed, the amount depending upon the density of the population to be counted. For extracting corn leaf aphids from sorghum heads, where as many as 5,000 have been found per head, an aliquot of only one-sixth pint may be used satisfactorily.

While the sample is being agitated, a nylon screen is placed on the filtering surface of each Büchner funnel and sprayed lightly with water so that it will seal tightly against the perforations. After an aliquot has been withdrawn, it is poured into the funnel. The nylon screen is then removed and placed on a wet masonite disk for counting under a binocular microscope. Wetting the disk enables the nylon to be floated on smoothly and quickly, and the wet surface keeps the insects in excellent condition until the counts are completed.

Extraction of Corn Leaf Aphids.—A number of tests were conducted to determine the efficiency of this method for extracting corn leaf aphids from the whorls and heads of sorghum. This insect occurs in tightly compacted whorls, and counts are usually made by peeling the leaves back individually. In these tests they were made in one of three ways: (1) by cutting the whorl into 3-inch sections and unrolling the leaves before placing them in the liquid, (2) by cutting the whorl into 3-inch sections and not unrolling, or (3) by neither cutting nor unrolling the whorls. Tests were also conducted with long and medium strokes of the agitator arm. After 5 minutes of agitation 1-pint aliquots were removed and passed through the nylon screens. The number of aphids per aliquot was determined, and also the insects remaining in the gallon jars. When the whorls were cut but not unrolled, the following percentages of aphids were recovered: long stroke 13.8 ± 1.0, medium stroke 13.0 ± 1.2. When the whorls were cut and unrolled, and agitated with a long stroke, the recovery was $18.3 \pm 2.9\%$. Whorls placed in baskets without cutting or unrolling and agitated with a medium stroke had a recovery of $12.7 \pm 3.7\%$.

After each sample was extracted, the leaves were thoroughly agitated in detergent water and all remaining aphids screened and counted. Only when the whorls were neither cut nor unrolled were over 0.1% of the aphids found adhering to the leaves, and then only 3.5%. For extracting aphids from the whorls it was decided to cut the whorls in 3-inch sections without unrolling them.

Aphids were also extracted from sorghum heads, ⅛-pint aliquots being used for this purpose. In eight samples the recovery was $2.36 \pm 0.33\%$. The recovery per

pint, 14.2±2.0%, was close to the recoveries from the 1-pint aliquots.

Extraction of Corn Earworm Eggs.—When the fins were modified to increase the downward movement of the light corn silks, which otherwise had a tendency to mat on top of the liquid, the method proved to be satisfactory for estimating populations of corn earworm eggs adhering to corn silks.[3] A little chlorine water placed in the water instead of detergent appeared to loosen the eggs from the silks. The cylindrical baskets were lined with 16-mesh galvanized screen to retain the silks.

Extraction of Date Mites.—These mites are found in the crowns of wheat plants, where they are difficult to count. Ten plants about 4 inches high were placed in the churn jar and agitated in the same manner as the sorghum samples. The mites were counted directly on the nylon screens, at a magnification of 14 diameters.

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[3] Modification by K. D. Arbuthnot, Entomology Research Division, Stillwater, Oklahoma.

Effects of Heterogeneous Distribution and Codistillation on the Results of Tests with DDT Against Mosquito Larvae[1]

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ABSTRACT

The concentration of DDT in suspensions was shown to be less than the theoretical, and to vary with the volume of the suspension and the size of the container. This variation resulted from differences in the loss of DDT by codistillation and/or by association with the water interfaces, which explained the resulting differences in mosquito mortality. The mortality of *Anopheles quadrimaculatus* Say was influenced more than that of *Aedes aegypti* (L.), owing to a difference in the behavior of the larvae and the dosage-mortality response. Biological data obtained with parathion, malathion, lindane, and dieldrin indicate that insufficient quantities of these insecticides are lost from the containers to alter mortality under normal test conditions.

Laboratory tests in which mosquito larvae are exposed in aqueous suspensions or solutions are commonly used in larvicide evaluation programs and resistance studies. In tests with DDT many workers have shown that the larval mortality at a given concentration and exposure time varies with such factors as the volume of suspension, the number of larvae per container, and the size and type of the container (Schmidt & Weidhaas 1959). There has been no completely satisfactory explanation of this phenomenon. Cutkomp (1947) reported that DDT was found on the sides and bottoms of glass jars containing dilute dispersions that had been aged and aerated. Kruse *et al.* (1952) considered that the mortality of larvae of *Anopheles quadrimaculatus* Say was lower in paper containers than in glass because the DDT "settled out" faster in paper containers. They also suggested that the total available DDT per larva and the "mass-surface area relationship per larva" might be responsible for the variation in mortality response with different test methods. On the basis of the results obtained in these two papers, Hawkins & Kearns (1956) made further studies and reported that the difference in mortality in paper and glass containers was correlated with the zeta potential and flocculation behavior of DDT suspensions.

Recently Bowman *et al.* (1959) showed that no settling of DDT occurred in aqueous suspensions at 0.01 p.p.m., but some did occur at 1.0 p.p.m. Furthermore, they showed that the distribution of DDT in the water was not homogeneous. Immediately after the preparation of 0.01-p.p.m. suspensions in glass jars, the DDT concentrated in the water interfaces, and in 24 hours more than 50% of it was lost by codistillation with water vapor. More DDT was lost by codistillation from paper than from glass containers, which would account for the lower mortality in paper containers. Consequently, it appeared that the explanation for the differences in mortality based on flocculation and settling of DDT was not valid for test concentrations in the order of 0.01 p.p.m. In addition, the heterogeneous distribution and loss of DDT from all types of containers supplied a possible explanation for the variation in mortality of mosquito larvae with different testing techniques.

The experiments reported in this paper were designed to show the relationships between the mortality of mosquito larvae and the distribution and loss of DDT under different test conditions. DDT labeled with carbon-14 was used in all experiments unless otherwise noted. Suspensions were prepared by adding acetone solutions to water. The term "theoretical concentration" is used to indicate the concentration that would have been obtained if all the DDT introduced were equally distributed in the suspension. In addition, biological tests were conducted with dieldrin, lindance, malathion, and parathion to determine if similar relationships existed.

DISTRIBUTION OF DDT IN TEST JARS.—In some experiments the distribution of the DDT was determined when the theoretical concentration, the volume of suspension, and the quantity of acetone used to introduce the DDT were varied. No larvae were present, but all other conditions were similar to those used in biological tests. In other experiments the distribution of DDT was determined in containers of different sizes with larvae present. The experimental techniques and the radiometric methods of analysis described by Bowman *et al.* were used. All results presented are the averages of duplicate tests.

[1] Accepted for publication August 10, 1959.
[2] H. R. Ford assisted in these experiments.

Table 1.—Distribution of DDT in aqueous suspensions in open glass jars 2 minutes and 24 hours after preparation at three concentrations.

Theoretical Concentration (p.p.m.)	DDT Recovered			
	In Suspension		Jar Surface (%)	Total (%)
	P.p.m.	Per Cent		
	After 2 Minutes			
0.004	0.0034	85	12	97
.007	.0062	88	16	104
.01	.0082	82	11	93
	After 24 Hours			
0.004	0.0012	30	7	37
.007	.0018	25	9	34
.01	.0033	33	7	40

The distribution of DDT in glass jars (79 mm. diameter) 2 minutes and 24 hours after preparation at concentrations of 0.004, 0.007, and 0.01 p.p.m. is shown in table 1. Approximately 60% of the DDT was lost from the jars by codistillation during 24 hours. Some DDT was found on the inner surfaces at 2 minutes and smaller amounts after 24 hours; at both intervals the amounts were lower than those reported by Bowman *et al.* (7% to 16% as against 13% to 39%). The reason for this difference is unknown. The respective concentrations in suspension were about 80% and 30% of the theoretical.

The distribution of DDT when the volume of the suspension was varied is shown in table 2. At 2 minutes a higher percentage of DDT was found on the jar surface with 100 ml. than with 250 or 500 ml., although the amount increased with the volume. The per cent lost by codistillation in 24 hours decreased as the volume increased, although the amount lost increased. The loss of DDT to the water interfaces and by codistillation resulted in a lower concentration in the smaller volumes than in the larger volumes.

Since the amount of acetone used to prepare the suspensions might affect the distribution of DDT, 250-ml. volumes were prepared at a concentration of 0.007 p.p.m. with 0.1% and 2% of acetone. The DDT in suspension and on the inner surface of the jar was determined at 2 minutes and 24 hours in open jars and at 72 hours in jars covered with aluminum foil. As shown in table 3, there was no significant difference in distribution with the two quantities of acetone, but covering the jars reduced the loss of DDT greatly.

Table 2.—Distribution of DDT at a theoretical concentration of 0.007 p.p.m. in different volumes of aqueous suspension in open glass jars 2 minutes and 24 hours after preparation.

Volume[a] (Ml.)	DDT Recovered			
	In Suspension		Jar Surface (%)	Total (%)
	P.p.m.	Per Cent		
	After 2 Minutes			
100	0.0054	79	30	109
250	.0062	88	16	104
500	.0058	83	14	97
	After 24 Hours			
100	0.0011	16	8	24
250	.0017	25	9	34
500	.0026	37	6	43

[a] 79-mm. jars with 100 and 250 ml. and 85-mm. (600-ml.) beakers for the 500-ml. volume.

Table 3.—Distribution of DDT at a theoretical concentration of 0.007 p.p.m. in aqueous suspensions prepared with different quantities of acetone in open or closed glass jars at various intervals after preparation.

Location of DDT	Per Cent of DDT Recovered	
	0.1% Acetone	2% Acetone
After 2 Minutes, Open Jars		
Suspension	77	73
Inner surface of jar	21	25
Total	98	98
After 24 Hours, Open Jars		
Suspension	30	33
Inner surface of jar	15	11
Total	45	44
After 72 Hours, Closed Jars		
Suspension	65	68
Inner surface of jar	19	20
Total	84	88

The distribution of DDT was determined in jars of two sizes—the regular jars 79 mm. and battery jars 152 mm. in diameter. Each jar contained 250 ml. of suspension at a concentration of 0.007 p.p.m., and 25 *quadrimaculatus* larvae. After 24 hours the mortality was recorded and 20-ml. samples of the suspensions were withdrawn by pipette from the top and bottom of the jars. 1 100-ml. sample comprising the remainder of the top half of the suspension was also taken. The larvae then were removed, washed, ground, and analyzed according to the method previously reported (Schmidt & Weidhaas 1958). The pipetted samples and the suspension remaining in the jars were extracted with benzene, and an aliquot of each was plated and counted for radioactivity. The sides and bottoms of the jars were washed with 25-ml. portions of acetone, and an aliquot was analyzed.

The distribution of DDT is given in table 4. Fifty per cent of the mortality occurred in the regular jars but only 11% in the battery jars. After 24 hours the loss was greater in the battery jar and the amount of DDT found on the inner surface of this jar was twice that on the inner surface of the regular jar, whereas the reverse was true regarding the concentration of the suspension.

Biological Tests with DDT.—Experiments were run to correlate the larval mortality with the data on the distribution of DDT. Twenty-five fourth-instar larvae

Table 4.—Distribution of DDT in larvicidal tests after 24 hours when *Anopheles quadrimaculatus* larvae were exposed to a theoretical concentration of 0.007 p.p.m.

Location of DDT	Regular Jar		Battery Jar	
	μg.	Per Cent	μg.	Per Cent
Larvae	0.154	9	0.153	9
Top half of suspension	.240[a]	14	.110[a]	6
Bottom half of suspension	.256[a]	15	.130[a]	7
Sides and bottom of jar	.111	6	.209	12
Total	.761	44	.602	34

[a] Amount determined both by subsampling and total extraction.

were used in each test and the mortality was recorded after 24 hours. The stock solutions of C^{14}-DDT prepared for distribution studies were used in these tests; each test was replicated 2 to 4 times.

The mortality of *quadrimaculatus* larvae exposed to both labeled and unlabeled DDT was determined when the theoretical concentration was varied from 0.004 to 0.01 p.p.m. in 250 ml. of suspension and when it was constant (0.007 p.p.m.) and the volume was varied from 100 to 1,000 ml. Mortality varied with both the concentration and the volume, but there was no appreciable difference with labeled and unlabeled DDT.

To determine if the loss of DDT by codistillation influenced the mortality, *quadrimaculatus* larvae were exposed for 24 hours in 250-ml. suspensions at a concentration of 0.007 p.p.m. of DDT. One set of test jars was sealed with aluminim foil, a second set was left unsealed, and a third set, also unsealed, was placed in a 12-m.p.h. wind from a fan to increase the loss of DDT by codistillation. Loss of DDT had a pronounced effect on the mortality; it was greatest (96%) in the jars sealed with foil, less (59%) in the unsealed jars, and least (6%) in the jars over which air was blown. On the other hand, *Aedes aegypti* (L.) larvae exposed to 0.0025 p.p.m. showed no difference in mortality between sealed and unsealed jars (74% and 76%). A possible reason for this phenomenon is considered in the discussion.

A. quadrimaculatus larvae were exposed to various volumes of suspensions in open and sealed containers of different sizes. The loss of water through evaporation was measured by weighing the jars at the beginning and end of each test period. As shown in table 5, mortality was always higher in sealed than in open containers of the same size when the volume of suspension was the same. Schmidt & Weidhaas (1959) reported that the per cent mortality in open jars increased with the test volume and decreased with the container size. In these tests sealing jars with aluminum foil decreased, but did not completely eliminate, this variation in mortality. Water lost by evaporation from open jars was proportional to the diameter. This test clearly showed that loss of DDT by codistillation was partly responsible. A small amount of water was lost from the sealed containers; however, calculations based on the work of Bowman *et al.* showed that the amount lost could not remove sufficient DDT from suspension to affect the mortality. This test and calculations indicated that the association of DDT with the glass-, air-, or glass-air-water interfaces, as well as the loss by codistillation, had an effect on mortality.

To confirm the effect of the association of DDT with interfaces on larval mortality, *quadrimaculatus* larvae were exposed to 250 ml. of suspension at a concentration of 0.007 p.p.m. in open and closed 79 mm. (diameter) regular jars, 140 mm. petri dishes, and 152 mm. battery jars. The containers were sealed with greased glass covers. Only a small portion of the battery jar was occupied by the suspension, whereas the petri dish was almost full. The respective mortalities were 94%, 44%, and 50% when the containers were sealed, but only 72%, 16%, and 4% when they were open. The association of DDT with the various water interfaces was shown to have an adverse effect on the mortality, since it was lower in the closed battery jar and petri dish than in the regular jar.

Table 5.—Mortality of *Anopheles quadrimaculatus* larvae and loss of water in 24 hours in closed and open jars of different sizes or containing various volumes of DDT suspension (0.007 p.p.m.).

Diameter of Jar (Inches)	Volume of Suspension (Ml.)	Per Cent Mortality		Water Evaporated (Ml.)	
		Open Jar	Sealed Jar	Open Jar	Sealed Jar
		Jar size varied			
3	250	85	98	5.4	0.1
3.25	250	80	97	6.1	.1
4	250	79	93	7.4	.1
6	250	17	50	13.8	1.1
		Volume varied			
3	100	32	82	4.8	0.1
	200	82	95	5.0	.3
	250	90	97	4.6	.3
3.25	500	96	98	6.5	.4
4	1,000	98	99.5	11.4	.8

It seemed possible that preparing suspensions in bulk, as is sometimes done in testing procedures, then pouring aliquots into jars, could give different mortalities than preparing them directly in the test jars, because of the interface action. To test this possibility, 1 liter of stock suspension (0.07 p.p.m.) was prepared and then poured into another liter beaker, and a 250-ml. sample was taken from the second beaker and tested against fourth-instar *quadrimaculatus* larvae. The mortality obtained with this sample was compared with that obtained with samples from stock suspensions that had two or three successive pourings into liter beakers. Each transfer was made carefully to reduce to a minimum the disturbance of insecticide particles that might have adhered to the glass. Fifteen minutes were allowed between each transfer. There was no appreciable difference with samples from the first (88%), second (74%), and third (88%) pourings. In a similar test the distribution of DDT was determined radiometrically, but after the second pouring the suspension was divided equally into four regular jars. In one series of determinations transfers were accomplished by pouring as in the biological test mentioned above and in another by pipetting as in the distribution studies. When the suspension was poured, 4.4% of the DDT was found on the inner surface of the first beacker, 0.9% and 0.7% on the second and third beakers, and a total of 0.9% on the inner surfaces of the four jars. When transfers were made by pipetting these values were 6.0%, 5.0%, 6.2%, and 3.6%.

A. quadrimaculatus and *aegypti* larvae were exposed to suspensions of DDT in glass (79 mm.) and aluminum (85 mm.) containers which were left open or sealed with aluminum foil, *quadrimaculatus* larvae to 0.007 p.p.m. and *aegypti* to 0.0025 p.p.m. The mortality of the former was greater in the sealed glass jars (84%) than in the open ones (72%), and the mortality in aluminum containers was much lower in both open (5%) and closed (9%) containers than in glass jars. With *aegypti* larvae there was no difference between open and closed containers, and the mortality was only slightly lower in aluminum containers (89%) than in glass jars (95–96%

BIOLOGICAL TESTS WITH OTHER INSECTICIDES.—To determine whether codistillation influenced larval mor-

Table 6.—Mortality of *Anopheles quadrimaculatus* larvae exposed to 250 ml. of suspensions or solutions of four insecticides in regular jars sealed with aluminum, open, or open and placed in 12-m.p.h. air movement from a fan.

Insecticide and Theoretical Concentration (p.p.m.)		Per Cent Mortality at 24 Hours		
		Sealed Jars	Open Jars	Open Jars With Air Movement[a]
Dieldrin	0.10	—	60	22
	.075	55	53	—
	.04	—	18	0
Lindane	0.10	—	84	80
	.075	40	46	—
Parathion	0.008	—	100	100
	.006	—	74	80
	.004	98[b]	98[b]	—
Malathion	0.10	—	100	98
	.075	—	60	80
	.05	84[b]	90[b]	—

[a] Water evaporated ranged from 25 to 50 ml. per jar.
[b] Mortality at 48 hours, since there was no appreciable mortality at 24 hours

tality with dieldrin, lindane, parathion, and malathion, fourth-instar *quadrimaculatus* larvae were exposed to these insecticides in regular jars, which were sealed with aluminum foil, open, or open and placed in a 12-m.p.h. air current from a fan. Table 6 shows that with lindane, parathion, and malathion there was no appreciable difference under these conditions. It appeared that the loss of water due to the air current may have concentrated the parathion and malathion, since in one test mortality was greater in jars placed in the air current. The mortality was the same in open and sealed jars containing dieldrin, but less in jars placed in the air current, indicating that some dieldrin was lost. However, the quantities of the four insecticides that may have been lost from the containers were insufficient to alter the mortality under normal test conditions.

Discussion.—The biological and distribution data clearly showed that the loss of DDT, both by codistillation and by association at the water interfaces, influenced the mortality of *quadrimaculatus* larvae and was responsible for most of the variation with different test conditions. In all tests mortality was lower in open systems, from which a high percentage of DDT was lost by codistillation, than in closed systems. It was also lower when the standard test procedure was varied to favor codistillation and the loss to interfaces by increasing the diameter of the container and holding the volume constant or the percentage loss of DDT was favored by decreasing the volume in the same container. When sealed containers were used, preventing codistillation, the variation in mortality was not completely eliminated. The association of DDT at the water interfaces was probably responsible for the remaining variation, since the mortality of *quadrimaculatus* larvae was greater in glass than in aluminum containers. Bowman *et al.* showed that, although the loss by codistillation in 24 hours was similar from both types of containers, the loss to the water interfaces was much greater in aluminum.

Little is known about the association of DDT with the water interfaces or inner surface of containers. The amount of DDT found on the walls has varied considerably in distribution studies made under similar conditions at this laboratory. Analysis of the inner surfaces only showed the amount of DDT remaining on the walls after the suspensions were removed. This method does not necessarily characterize the dispersion before removal of the suspensions. In fact, the amount found on the walls differed when the suspensions were removed by pouring or by pipetting. However, analyses proved that all the DDT was not in the body of the suspensions.

Mortality of *aegypti* larvae was about the same in open and sealed glass and aluminum containers. Furthermore it has been shown (Schmidt & Weidhaas 1959) that variation in mortality under different test conditions was much less with *aegypti* than *quadrimaculatus*. One reason for this difference is that the two species exhibit a different relationship between the absorbed dose and mortality. Although the dose absorbed over time at a given concentration was similar for both species, the rate of increase in mortality was much greater for *aegypti* (Weidhaas & Schmidt 1960). This indicates that loss of DDT from test jars would have less effect with this species. In addition, *aegypti* attempt to feed along the walls of containers where apparently DDT tends to concentrate, particularly with aluminum.

Previous studies showed that it was possible to correlate mortality with the volume of test suspension, the number of larvae per container, the ratio of volume to number of larvae, and the total available DDT per larva (Schmidt & Weidhaas 1958, 1959). However, these correlations could not explain the variations in mortality with test conditions. Data in this paper show clearly that, except for the number of larvae per container, these correlations depended on the behavior of DDT suspensions. When the number of larvae was increased the larvae removed enough DDT to account for the reduction in mortality.

Until recently it was generally believed that larvae are exposed to a fairly homogenous distribution of DDT. These tests showed that larvae are exposed to a heterogeneous distribution of DDT in which the concentration after 2 minutes is lower than the theoretical value and continues to decrease with time. In spite of this, the suspension concentrations determined for the three concentrations studied after 2 minutes and after 24 hours, are proportional to the theoretical values.

The variation in mortality of mosquito larvae with DDT under different test conditions results from a complexity of factors, some of which are related to the physical properties of DDT in suspension, others to the species of larvae.

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Organophosphate Systemics as Sprays and Feed Additives for Cattle Grub Control[1]

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ABSTRACT

Ronnel (formerly designated Dow ET-57, *O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate, Trolene,® or Korlan®) was administered orally to steers at three locations at two dosage levels in the feed; 15 mg./kg./day for 7 days, and 25 mg./kg./day for 6 days. Bayer 21/199 also called Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) was applied to steers at two locations as either a 0.5% suspension or emulsion at 250 pounds pressure. There was no difference in grub (*Hypoderma* sp.) control, weight gain, feed efficiency or carcass grades between treatments at each location. Grub control at the three locations ranged from 76.3 to 98.6%.

In recent years many papers have been published on the use of systemic insecticides for the control of cattle grubs (*Hypoderma* sp.). Methods of administering the insecticides include boluses, sprays, drenches, injections, and feed additives (Brundrett *et al.* 1957, Burns & Newson 1958, Crenshaw 1956, French *et al.* 1958, Raun & Herrick 1957, Roberts *et al.* 1958, Roth & Eddy 1955). The feedlot tests reported in this paper were made to gain more information on the use of feed additives and sprays under field conditions. They were carried out at three different locations.

LOCATION I.—The steers in this test were from a Montana ranch and averaged slightly over 700 pounds at the beginning of the trial on October 23, 1958. The 92 animals were divided into four groups of 23 head each according to weight, in order to reduce the variability between lots. Each animal was identified with a neck chain at the time it was weighed.

The four feed lots in which the cattle were placed were identical and the cattle were handled in the same manner except for the experimental grub control treatments. Each lot of cattle received one of the three grub treatments, except lot A which was kept as a control unit.

Lot B steers received ronnel at the rate of 25 mg. per kg. per day for 6 days. Four and one-half pounds of 10% ronnel were mixed with the entire day's feed. This mixture was then placed in feed bunks which would accommodate all 23 head at one time.

Each animal in lots C and D received an application of 1 gallon 0.5% Bayer 21/199 also called Co-Ral®(*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) at a pressure of 250 pounds per square inch. Cattle in lot C were sprayed with a water emulsion of Bayer 21/199 while cattle in lot D were treated with a suspension of the test insecticide.

All cattle remained on test for 162 days and then were sold for slaughter, after individual weights were taken for calculations of weight gain and feed conversion. The animals were slaughtered on April 3, 1959, and grub counts were taken on the killing floor in the packing plant. Grubs on the carcass after the hide was removed were totaled with grubs found on the underside of the hide to calculate the grub control obtained by the various treatments.

Information on selling price, margin per steer over feed cost, dressing percentage, carcass grades, and condemned livers was obtained through the cooperation of the packing plant. (Table 1.)

There was no difference in grub control, dressing percentage, carcass grades, and condemned livers among the three methods of chemical application. (Table 1.) The average grub control was 77%.

The margin per steer over feed costs was largely in favor of the treatments, except for lot D, which made less margin per steer than the untreated cattle in lot A. This can be explained because the cattle in lot D had a mild outbreak of pneumonia during the test period, which threw them off feed for several days. Likewise the differences in rate of gain can be explained on this basis.

LOCATION II.—These cattle were from a Nebraska ranch. They were allotted to three different groups. Since this experiment was superimposed over a feeding experiment, equal numbers could not be used for each treatment. Sixteen head received ronnel mixed with corn meal added to the daily ration at the rate of 15 mg. per kg. body weight for 7 days. Twenty-four steers were sprayed with a 0.5% suspension of Bayer 21/199. A household detergent was added to the spray suspension at the rate of ½ pound per 25 gallons. The treatment was applied at a pressure of 250 p.s.i. Twelve head were kept as controls. All animals were weighed at monthly intervals throughout the 121 day test period, and there were no significant differences in rate of gain between treatments.

Grub counts were made on the carcasses and hides when the cattle were slaughtered. These counts indicated that ronnel in the feed for 7 days at the rate of 15 mg. per kg. per day gave 91% control of the grubs. The spray

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Table 1.—Systemic insecticides applied as suspension or emulsion sprays or mixed with the feed for control of cattle grubs at Location I. October 23, 1958.

Treatment	No. of Steers	Grub Control (%)	Average Daily Gain (Lb.)	Feed Cost/100 Pounds Gain	Dressing (%)	Carcass Grade	Condemned Livers
Lot B							
Ronnel mixed with feed for 6 days. (25 mg./kg./day)	23	76.3	2.6	$18.37	61.8	1 prime 19 choice 3 good	8
Lot C							
Bayer 21/199, 0.5% water emulsion spray at 250 p.s.i.	23	78.1	2.6	$17.69	62.3	20 choice 3 good	5
Lot D							
Bayer 21/199, 0.5% suspension spray at 250 p.s.i.	23	76.3	2.4	$19.14	61.8	20 choice 3 good	4
Lot A							
Untreated	23	0.0[a]	2.6	$17.47	61.2	17 choice 6 good	3

[a] Average 3.25 grubs per steer.

application of Bayer 21/199 controlled 98.6% of the grubs. (Table 2.)

There appeared to be a palatability problem in connection with the use of ronnel as a feed additive. The cattle consumed 25% less feed the first 3 days after the insecticide was mixed with it. Since this did not appear to be a problem at Location I where the insecticide was mixed with a soybean meal feed supplement at a dosage 10 mg. per kg. per day higher, it may be that some masking supplement will eliminate the palatability problem.

LOCATION III.—Three hundred and forty-eight Montana steers from three different ranches were gate-cut on arrival at the feedlot. One hundred and seventy head were placed in one lot and 178 in the other. The average initial weight of animals in both lots was 558 pounds.

On December 10, 1958, the 178 animals in one lot began a 7-day treatment with ronnel mixed with corn meal and added to the daily ration. The dosage was 15 mg. per kg. per day. These cattle also evidenced a dislike for the feed mixture during the first 3 days of the feeding period.

On February 26, 1959, and again on April 9, grub counts were taken on a random basis on 30 steers from the treated lot and 30 steers from the untreated lot. Counts were made by palpation (*e.g.*, running the hand over the animal's back and counting grub cysts while the steer was held in a restraining chute). These grub counts indicated that ronnel gave 97% control in this test. (Table 2.)

One steer from the ronnel-fed cattle became paralyzed during the last day of the feeding period. After symptomatic treatment seemed to have no desirable effect the animal was sacrificed. No reason for the paralysis could be found. The brain, spinal cord, liver, spleen and kidneys appeared perfectly normal. No grub larvae were found in any of the vital organs.

Table 2.—Systemic insecticides applied as suspension sprays or mixed with the feed for control of cattle grubs.

Treatment	No. of Steers	Grub Control (%)
Location II, October 14, 1958		
Ronnel mixed with feed for 7 days (15 mg./kg./day)	16	91.0
Bayer 21/199, 0.5% suspension spray with ½ lb. detergent added per 25 gal. at 250 p.s.i.	24	98.6
Untreated	12 (Av. 11.0 grubs/steer)	0.0
Location III, December 10, 1958		
Ronnel mixed with feed for 7 days (15 mg./kg./day)	178	97.0
Untreated	170	0.0[a]

[a] Average 19.6 grubs per steer.

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O-Ethyl *S*-2-(Ethylthio)-ethyl Alkylphosphonothioates as Systemic Insecticides[1,2]

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Abstract

The contact and systemic insecticidal activities of 10 alkylphosphonate esters containing the 2-(ethylthio)-ethyl group were determined. Some of these compounds were highly toxic by contact and were especially effective systemic insecticides when applied topically or by seed treatment to cotton plants. The relations of structure to activity were in accord with those to be expected from previous work on related series of compounds and as predicted from theoretical grounds.

In previous papers from this laboratory, the insecticidal properties of *O*-alkyl *O*-*p*-nitrophenyl alkylphosphonate and phosphonothioate esters have been studied (Fukuto & Metcalf 1959a, Fukuto *et al.* 1959). Many of these compounds proved to be extremely toxic to insects and it became of interest to explore the possibilities of utilizing displaceable groups other than *p*-nitrophenoxide moiety. The remarkable systemic activity of demeton or Systox (Trademark, Chemagro Corp.) (*O,O*-diethyl *O*(*S*)-2-(ethylthio)-ethyl phosphorothioate) isomers is well known and attention was therefore directed to alkylphosphonates, in which the displaceable moiety was 2-(ethylthio)-ethanethiol. Since the initiation of this work several years ago, one of these compounds, *O*-ethyl *S*-2-(ethylthio)-ethyl methylphosphonothiolate, has been described as an active systemic insecticide by Paikin *et al.* (1957). The importance of optical activity in the toxicity of compounds of this series, *i.e.*, *O*-ethyl *S*-2-(ethylthio)-ethyl ethylphosphonothiolate has been studied by Aaron *et al.* (1958) and Fukuto & Metcalf (1959b) have shown that the *l*-isomer of this compound is 6 to 10 times as toxic to insects as the *d*-isomer. This paper describes the preparation, properties, and preliminary evaluation of the insecticidal activity of typical compounds of this type.

Materials and Methods.—The preparation of some of the compounds studied in this paper is described by Fukuto & Metcalf (1959a and 1959b). The properties and analyses of the new compounds are listed in table 1.

O-Alkyl S-2-(ethylthio)-ethyl alkylphosphonothiolate.—The compounds in table 1 were prepared according to Fukuto & Metcalf (1959a) from 2-(ethylthio)-ethanethiol or 2-(ethylthio)-ethanol and the appropriate alkyl alkylphosphonochloridate.

O-Ethyl S-2-(ethylsulfinyl)-ethyl n-propylphosphonothiolate.—*O*-Ethyl *S*-2-(ethylthio)-ethyl *n*-propylphosphonothiolate previously described by Fukuto & Metcalf (1959a) was oxidized to the corresponding sulfoxide by hydrogen peroxide according to Fukuto *et al.* (1955).

The topical LD_{50} values to the house fly, *Musca domestica* (L.), were determined by the application of 1-microliter droplets of acetone solution to the thoraces of groups of 20 female flies, and the values given are the averages of three determinations. The systemic activity was measured by the topical application of 5 microliters of pure compound to the stems of young cotton plants about 25 cm. high and subsequent bioassay of isolated leaves in Munger cells over a 24-hour period with 20 adult female greenhouse thrips, *Heliothrips haemorrhoidalis* (Bouché), 20 adult female two-spotted spider mites, *Tetranychus telarius* (L.), or fifth-instar cotton leaf perforator larvae, *Bucculatrix thurberiella* Busck. For the experiments with seed treatment, 5 microliters of compound and 5 mg. of powdered charcoal were mixed in one-half of a "No. 1" gelatin capsule and a cotton seed was placed in the half-capsule, which was then planted upright. Leaves were removed at intervals and assayed as with the topical treatments. Cholinesterase inhibitory activity of the compounds in acetone solution was determined manometrically using house fly head enzyme as described by Metcalf & March (1949). The anticholinesterase activity of cotton leaf tissues containing systemically translocated materials was determined by homogenizing a weighed amount of leaf in distilled water and pipetting 0.1 ml. of the homogenate containing the desired weight of leaf tissue directly into the Warburg flask.

Discussion of Results.—*Contact toxicity and cholinesterase inhibition.*—The contact toxicity of the compounds studied to *Musca domestica* is given in figure 1, and it is clear that certain of these alkylphosphonate esters are highly active insecticides. The activity in the phosphonothiolate series decreased with the increase in length of the alkylphosphonate group from methyl > ethyl > propyl > isopentyl. A similar effect of chain length on toxicity was recently reported for a corresponding series of *O*-ethyl *O*-*p*-nitrophenyl alkylphosphonates (Fukuto & Metcalf 1959a) and is to be expected from the inductive and steric effect of the alkyl groups in decreasing the electrophilic character of the phosphorus atom and consequently lowering the reactivity of the molecule to nucleophilic agents. This effect is correlated with Taft's polar substituent constant σ^* which appears to approximate the magnitude of the effects observed (Taft 1953).

It is of interest to compare the toxicity of the series of compounds described here in which 2-(ethylthio)-ethanethiol is the displaceable group in the bimolecular reaction with cholinesterase enzyme (Fukuto 1957) with the companion series of phosphonates in which *p*-nitrophenol is the displaceable group (Fukuto & Metcalf 1959a, Fukuto *et al.* 1959). A comparison of the activities of the two sets of compounds is, however, complicated by the well known *in vivo* oxidation of the sulfide group to sulfoxide and sulfone (Fukuto *et al.* 1955, Metcalf *et al.* 1957). A comparison of the *in vitro* activity of the compounds as inhibitors of fly brain cholinesterase is more indicative of intrinsic reactivity. From a comparison of both toxicity and enzyme-inhibitory activity of the compounds in figure 1 with those described in tables 2 and 4 of Fukuto *et al.* (1959), it is clear that the ratios of

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Table 1.—Properties and analysis of new compounds investigated in this paper.

Compound	b.p. ° C. Phosphonochloridate Intermediate	Compound	Elemental Analysis[a] Calculated	Found
$(CH_3)_2CH_2CH_2CH_2P(O)(OC_2H_5)SCH_2CH_2SC_2H_5$	95–104 (7 mm.)	108–12 (0.1 mm.)	C 46.5 H 8.85	C 44.84 H 9.42
$C_2H_5P(S)(OC_2H_5)OCH_2CH_2SC_2H_5$	76–80 (10 mm.)	105 (0.1 mm.)	C 39.65 H 7.90	C 39.09 H 8.65
$C_2H_5P(S)(OC_2H_5)SCH_2CH_2SC_2H_5$	76–80 (10 mm.)	108 (0.3 mm.)	C 37.18 H 7.41	C 37.36 H 7.98
$C_2H_5P(O)(OC_2H_5)OCH_2CH_2SC_2H_5$	80–86 (15 mm.)	78–82 (0.1 mm.)	C 42.46 H 8.46	C 41.60 H 9.29
$C_2H_5P(O)(OCH(CH_3)_2)SCH_2CH_2SC_2H_5$	73–78 (10 mm.)	94–96 (0.1 mm.)	C 42.2 H 8.26	C 41.06 H 8.41
$C_2H_5P(O)(SCH(CH_3)_2)SCH_2CH_2SC_2H_5$	76 (0.6 mm.)	118 (0.07 mm.)	C 39.7 H 7.78	C 39.37 H 7.98
$C_2H_5P(O)(OC_2H_5)SCH_2CH_2S(O)C_2H_5$	84–92 (12 mm.)	140 (0.1 mm.)	C 39.7 H 7.77	C 39.75 H 8.29

[a] Microanalyses by C. F. Geiger, Ontario, California.

the LD_{50} values of the 2-(ethylthio)-ethanethiol esters to those of the *p*-nitrophenyl esters is remarkably constant: methylphosphonates 7.0, ethyl 7.9, *n*-propyl 5, isopentyl 5.7; and that the ratios of I_{50} values for cholinesterase inhibition are somewhat larger, methyl 15.5, ethyl 7.8, *n*-propyl 12.0, and isopentyl 35.0, but of the same general magnitude. This conclusion suggests for the 2-(ethylthio)-ethanethiol esters not only some metabolic enhancement of toxicity in the insect, *i.e.*, the *in vivo* oxidation to sulfoxide and sulfone to produce compounds of greater reactivity, but also that toxicity and cholinesterase inhibition are closely related as has been demonstrated for a number of other groups of organophosphorus esters (*e.g.*, Fukuto & Metcalf 1956).

In the case of the phosphonothionate esters the I_{50} values are of little utility in measuring toxicity because of the *in vivo* oxidation of P=S to P=O (Metcalf & March 1953, Metcalf *et al.* 1957) which enhances reactivity 10- to 100-fold and the P=S and P=O esters are of roughly equivalent toxicity (fig. 1). Here again the 2-(ethylthio)-ethanethiol esters are only about 0.1 as active as enzyme inhibitors as the *p*-nitrophenyl esters.

When the displaceable group becomes 2-(ethylthio)-ethanol the toxicity and reactivity of the compounds are very greatly decreased, as has been demonstrated for the corresponding Systox series (Schrader 1952, Fukuto *et al.* 1955).

Systemic activity.—The systemic activity of the compounds under study was evaluated by using cotton plants treated in two ways: (1) by the topical application of 5 microliters of the pure compound to the stem, and (2) by applying 5 microliters as 50% charcoal powder to the

FIG. 1.—Insect toxicological properties of compounds investigated. The bar graphs indicate the 24-hour per cent mortalities of two-spotted spider mite (left, gray), greenhouse thrips (center, light gray), or cotton leaf perforator (right, black) feeding on isolated leaves from cotton treated on stem or seed at the indicated days before assay.

Table 2.—Absorption and translocation of Systox thiol-isomer and alkyl phosphonate analogues following application of 5λ to cotton stem.

Compound	Per Cent ChE Inhibition from Mg. Terminal Leaf Tissue at Indicated Hours After Treatment						
	4 Hours			24 Hours			
	33 Mg.	1 Mg.	0.1 Mg.	1 Mg.	0.1 Mg.	0.01 Mg.	0.001 Mg.
$C_2H_5OP(O)(OC_2H_5)SCH_2CH_2SC_2H_5$	60	27	6	77	14	—	—
$CH_3P(O)(OC_2H_5)SCH_2CH_2SC_2H_5$	99	99	94	94	94	26	3
$C_3H_7P(O)(OC_2H_5)SCH_2CH_2SC_2H_5$	99	99	96	96	94	22	0

seed. The toxicity of the translocated substances in the leaves was measured at intervals up to 60 days after application. As literally hundred of observations were made using the three test organisms, the general systemic activities of the compounds can best be judged by the overall heights of the bar graphs of figure 1, representing the per cent mortalities of the test organisms, *T. telarius*, *H. haemorrhoidalis*, and *B. thurberiella*, after 24 hours' exposure on the treated leaves in Munger cells. In general the systemic effectiveness of the compounds was closely related to the contact toxicity. With the series of ethylphosphonates, the thiol-isomer (II) was markedly superior to the thiono-isomer (VIII) as in the case with the Systox isomers, and the thiono-thiol compound (VII) was especially active as a seed treatment as is the case with the dithio-analogue of Systox, *O,O*-diethyl *S*-2-(ethylthio)-ethyl phosphorodithioate or Di-Syston® (Chemagro Corp.). The high activity of *O*-ethyl *S*-isopropyl *S*-2-(ethylthio)-ethylphosphonodithioate (VI) is especially interesting since it was shown in a previous paper (Fukuto *et al.* 1959) that the corresponding *p*-nitrophenyl ester is very susceptible to aqueous hydrolysis. All the highly active systemic compounds (I, II, III, V, VI, VII, X) were effective against not only mites and thrips but also the *B. thurberiella* larvae.

The rapidity of absorption and translocation of the phosphonate esters is indicated by the data in table 2, which shows the amount of toxicant in known amounts of cotton leaf tissue was measured by its inhibitory action to fly head cholinesterase. The methyl- and propylphosphonates were compared with Systox thiol-isomer, *O,O*-diethyl *S*-2-(ethylthio)-ethyl phosphorothiolate (I_{50} 3.5×10^{-6} *M*). It will be seen that the amount of anticholinesterase activity in the leaves following the application of the phosphonates was much higher than with the phosphate. This is partially due to the 10- to 30-fold greater enzyme inhibitory power of the phosphonates (fig. 1), but the 50- to 100-fold greater anticholinesterase activities in the leaves indicate that the phosphonate esters are absorbed and translocated at least as rapidly as the Systox thiol-isomer. A quantitative evaluation of this experiment is difficult because of the oxidation of these compounds in leaf tissue to the sulfoxide- and sulfone-derivatives. These are more active (Metcalf *et al.* 1957), but it seems logical that, other factors being equal, the compounds resulting in the highest anticholinesterase activity in the leaves should be the most active systemic insecticides and this is confirmed by the systemic toxicity data available.

It is also interesting to compare the influence of the 2-(ethylthio)-ethanethiol group upon absorption and translocation of the phosphonates. In another experiment cotton plants were treated topically on the stem with 5 microliters of *O*-ethyl *S*-2-(ethylthio)-ethyl propylphosphonothiolate and with *O*-ethyl *O*-*p*-nitrophenyl propylphosphonate. Despite the fact that the latter compound was five times as toxic as the former by topical application to the house fly, the cotton leaves from the treated plants gave the following per cent mortalities to red spider mites in Munger cells: after 1 day 0%; 2 days 20%; 5 days 10%; 8 days 70%; 23 days 10%. In comparison, with *O*-ethyl *S*-2-(ethylthio)-ethyl propylphosphonothiolate, 100% mortalities were obtained throughout the 23-day period. This fact indicates the superior systemic properties imparted by the 2-(ethylthio)-ethanethiol group, as is the case with the corresponding diethyl phosphates.

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Observations on Soybean Insects in Maryland[1]

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ABSTRACT

In the summer and fall of 1958 surveys were conducted on the Eastern Shore and in Prince Georges County, Maryland, to determine the extent of injury by various soybean pests. Tests to determine the effectiveness of phorate (formerly designated Thimet,® *O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate), malathion, and DDT for the control of onion thrips, *Thrips tabaci* Lind., tobacco thrips, *Frankliniella fusca* (Hinds) and *Sericothrips variabilis* (Beach); spider mites, *Tetranychus* spp., and corn earworm, *Heliothis zea* (Boddie), respectively, were also conducted.

Surveys indicated that arthropod populations on soybeans were low throughout the State in 1958, probably resulting from cool, moist weather conditions which appeared unfavorable to the growth and development of most common pests of this crop. The green cloverworm, *Plathypena scabra* (F.), was the most abundant injurious pest on soybeans, although its injury, along with that of corn earworm, spider mites and thrips, was not serious. The velvetbean caterpillar, *Anticarsia gemmatalis* Hbn., was not recorded in Maryland in 1958.

Chemical control tests showed that malathion, applied at the rate of 25 gallons per acre, resulted in a very noticeable increase over check plants in plant yields based on the weight of pods and beans. DDT and phorate treatments gave little or no increase. In all but one instance phorate treatments resulted in reduced yields, owing to low germination of treated seed.

Insect injury to soybeans in Maryland has been less serious than to many other crops such as fruits and vegetables. However, soybeans are replacing truck crops in some areas (Newcomer *et al.* 1954), and with the rapid increase in soybean acreage some yield reduction has resulted from insect damage. The work reported here was initiated to assess the damage to soybeans caused by insects and mites under varying conditions and to evaluate some insecticidal treatments.

In 1957 various kinds of insect injury to soybeans occurred throughout most of the growing season (Harding 1957). The two-spotted spider mite, *Tetranychus telarius* (L.) and the strawberry spider mite, *T. atlanticus* McG., caused heavy damage in some fields, particularly on the Eastern Shore during August. Considerable late season injury was also inflicted by the corn earworm, *Heliothis zea* (Boddie), which damaged pods in late August and early September.

In addition to these pests there were other insects that caused varying amounts of damage in 1957, and in certain areas they developed unusually high infestations. In June, bean leaf beetles, *Cerotoma trifurcata* (Forst.), were abundant in most sections of the State where soybeans were grown. Thrips, onion thrips (*Thrips tabaci* Lind.), tobacco thrips (*Frankliniella fusca* (Hinds)), and *Sericothrips variabilis* (Beach), caused some early season leaf drop on the Eastern Shore. General infestations of green cloverworm, *Plathypena scabra* (F.), on the Eastern Shore and in Southern Maryland during August, caused losses to some growers. Japanese beetles, *Popillia japonica* Newm., were reported doing some damage in July. An unusual outbreak of velvetbean caterpillars, *Anticarsia gemmatalis* Hbn., in Southern Maryland in August, caused severe damage to one 50-acre field in Prince Georges County. The clover root curculio, *Sitona hispidula* (F.), and the potato leafhopper, *Empoasca fabae* (Harr.), had also been injurious prior to 1957.

Relatively little detailed information has been published on soybean pests in the United States. Balduf, (1923), who collected 209 species of arthropods on soybeans made one of the earliest studies. Kulash (1947) noted that the velvetbean caterpillar and the corn earworm were important soybean pests in North Carolina. In Minnesota Kretzschmar (1948) found the potato leafhopper to be the most abundant soybean insect. Packard (1951) pointed out the importance of many insect pests in soybean production. Nettles (1958) and Milliron (1958) reported on soybean pests in South Carolina and Delaware respectively. The status of soybean insects in nineteen States and one province of Canada with control recommendations has been reviewed (Anonymous 1957).

Reports of experimental work with insecticides for control of arthropod pests of soybeans have been made by Kulash (1947), Cox & Lilly (1952), Reynolds *et al.* (1957), Probst & Everly (1957a, b), and Nettles (1958).

SURVEYS IN 1958.—Attempts were made to obtain reasonable estimates of the extent of damage caused by the following pests: thrips, green cloverworm, spider mites, and corn earworm. These were considered of first importance. Methods of recording infestations were generally those reported for Cooperative Economic Insect Reports (U.S.D.A. 1955). Observations and collections were also made to assess damage by less important pests. Surveys were made in six Eastern Shore counties and in Prince Georges County at intervals from June to October.

Thrips. Injury by thrips begins to show when plants are small, and takes the form of silver spotting and streaking. Thrips may cause a tight curling of young leaves. Three species were identified,[2] tobacco thrips, onion thrips, and *Sericothrips variabilis*. Thrips caused considerable injury in Prince Georges County during the early part of the summer, but populations decreased sharply following heavy rains in July.

Green Cloverworm. Light infestations were noted in mid-July. In Prince Georges County there was no population increase. But populations increased on the Eastern Shore during August. In some fields there was a count of

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[2] Through the courtesy of Miss Kellie O'Neill, U. S. Department of Agriculture, Agr. Res. Serv., Entomology Research Division.

eight worms per foot of row—enough to warrant control measures, and several fields were treated with DDT. In mid-August the green cloverworm was the most abundant soybean insect on the Eastern Shore, but populations began to decline in late August partly because of a fungus disease.

Spider Mites. Two-spotted spider mites were found in Dorchester, Somerset, and Worcester Counties in mid-August. There was yellowing of leaves in some fields, and noticeable stunting. Abundant rain and cooler weather probably effected mite population decline in late August and early September. No mites were observed in Prince Georges County. Spider mites usually enter a field from one side, evidently originating from plants on the field borders. Border plants found infested with mites are red clover and lespedeza. It is presumed they are carried to the soybeans by water or air currents.

A typical case of spider mite injury was noted in a soybean field in Worcester County in early August. Plants had been killed along a paved road in an area 120 feet long and extending inward in an arc about eight rows wide in the middle. For another 12 or 15 rows plants were visably affected with bottom leaves dropping and many upper leaves losing color. Proceeding further into the field, the populations gradually decreased but mites could be found 50 rows in and also on the far side of the field. The whole area included about 6 acres.

Corn Earworm. There was little evidence of corn earworm injury throughout the summer. On September 10 a light infestation was found in one field in Dorchester County. This infestation was observed to be increasing on September 18 with appreciable injury to pods. Some damage occurred in a few other areas in Dorchester County. Most of the damage was to late maturing plants.

Other Insects. Infestations of the Japanese beetle were noticeable in many fields on the Eastern Shore and in Prince Georges County during June and July, but observations indicated that the actual damage was not severe enough to affect yields adversely. Later in the summer recovery from leaf feeding injury was rapid. Small numbers of bean leaf beetles were observed in some fields on the Eastern Shore in July and August. In late August a heavy infestation developed in one field where the plants were small. In other scattered areas conspicuous damage by the leaf beetle occurred in September. In Prince Georges County small numbers were seen, but there was no appreciable damage. Grasshoppers (chiefly the differential grasshopper, *Melanoplus differentialis* (Thos.)) did some damage in July and August, particularly in Dorchester County. Small numbers were observed in Caroline County in September.

The Mexican bean beetle, *Epilachna varivestis* Muls., was present in some fields on the Eastern Shore but appeared to cause no injury. In Prince Georges County there was conspicuous damage in the experimental plot. This will be mentioned later. Several other insects were observed feeding on soybeans, but they appeared to cause little or no damage. These were the tobacco flea beetle (*Epitrix hirtipennis* (Melsh.)), the three-spotted flea beetle (*Disonycha triangularis* (Say)), the toothed flea beetle (*Chaetocnema denticulata* (Ill.)), the elongate flea beetle (*Systena elongata* (Fabr.)), the Asiatic oak weevil (*Cyrtepistomus castaneus* (Roelofs)), the spotted cucumber beetle (*Diabrotica undecimpunctata howardi* Barber), the grape colaspis (*Colaspis flavida* (Say)), the potato leafhopper, and the tarnished plant bug (*Lygus lineolaris* (P. de B.)).

EVALUATION OF INSECTICIDES.—Three insecticides, phorate formerly designated Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate), malathion, and DDT, were used on an experimental plot at Aquasco, Prince Georges County. The plot included four replications, with each replication consisting of four rows. Rows measured 330 feet in length and were spaced 38 inches apart, giving a total area of 16,500 square feet or approximately 0.33 acre. Each replication consisted of treatments as follows: a phorate-Arasan 75 combination, a malathion spray, a DDT spray, and a check. The phorate-Arasan 75 was applied as a seed treatment prior to planting. The seed was treated in the laboratories of the American Cyanamid Company.

Treatment A consisted of phorate seed treatment as a 97% emulsifiable concentrate applied at the rate of 0.5 lb. per 100 lbs. of seed, plus Arasan 75 at the rate of 2 oz. per 100 lbs. of seed. This treatment was made to seed in the first row of replications 1 and 3.

Treatment B consisted of a seed treatment of phorate 44-D (phorate formulated as a 44% material on activated charcoal) applied at the rate of 0.5 lb. actual per 100 lbs. of seed, plus Arasan 75 at the rate of 1.33 oz. per 100 lbs. of seed. This treatment was made to seed in the first row of replications 2 and 4. The sticker used for treatments A and B was American Cyanamid formula number 24-1, comprised of 1.5 parts Aerotex Resin number 159, 1.5 parts polyethylene glycol 400 and 2 parts of water.

Treatment C consisted of one application of a malathion spray, consisting of 20 ml. of 57% emulsifiable concentrate in 1 gal. of water, applied at the rate of 25 gals. per acre (0.66 lbs. actual malathion per acre).

Treatment D consisted of one application of DDT spray, consisting of 76 ml. of 25% emulsifiable concentrate in 1 gal. of water, applied at the rate of 65 gals. per acre. All sprays were applied with a 2.5 gal. compressed air sprayer at a pressure of approximately 25 to 30 lbs.

The entire plot was planted on July 18, 1958, with a four-row corn planter adapted for planting soybeans. All rows in the fourth replication were planted at the rate of 62 lb. per acre, while all other replications were planted at the rate of 46 lb. per acre. Soybean plantings throughout the State, in general, were delayed in 1958 because of excessive rain. All rows were planted with the variety Wabash. This is a fairly early maturing variety, requiring from 118 to 133 days in which to mature (Newcomer *et al.* 1954). Germination tested by the Agricultural Experiment Station Seed Laboratory at the University of Maryland showed 73% germination of this seed.

One application only of malathion and DDT was made on each replication. The malathion spray was applied on August 16, 1958, to the second row of each replication. Plants were approximately 10 to 12 inches high at that time. The DDT spray was applied to the third row of each replication on September 20, 1958. At that time plants were approximately 2.5 feet high. Because of the size of the plants at the latter date, it was necessary to apply the spray to one side of the row at a

time in order to obtain adequate coverage. This application was delayed purposely because of the few insects feeding on the plants in this plot. It was applied at this time to give protection against the corn earworm and other leaf-feeding caterpillars.

Within a week after seed was planted a higher rate of germination was noted in the untreated rows. On July 24 a count of the plants in 25 feet of row of both treated and untreated rows in each replication indicated that there was an average of 52% more plants in untreated rows.

In early August Mexican bean beetle larvae were found throughout the field. On August 16 although mites were not in evidence, a malathion spray was applied hoping to prevent a population buildup. After this spray it appeared that Mexican bean beetle populations increased more rapidly on unsprayed plants than on sprayed plants, but infestations were not severe and appeared to have no detrimental effect on plant growth throughout the field.

On September 20 a DDT spray was applied for corn earworm control even though this insect had not been detected. It was not detected later. Careful studies of damage caused by the Mexican bean beetle were made in October. Leaves from 160 plants (10 per replication) were examined. The percentage of injured leaves ranged from 11.5 to 39.3; and the percentage of injury to leaves was observed to average less than 25. There were no indications of appreciable differences in the degree of injury to plants receiving the various treatments.

Plants were hand-harvested on December 21. The yield of plants in each treatment and check was based on the weight of pods and beans from plants in 30 feet of each row. These plants were harvested in three 10-foot sections of each row, the sections being selected at random along the length of the row. The data are presented in table 1.

From the data in table 1 it is evident that there was a very noticeable increase in yield from those plants which received the malathion spray on August 16 (treatment C). This fact is attributed primarily to the reduction of Mexican bean beetle infestation in mid-August. Other injurious insects present at that time, such as thrips, green cloverworm and grasshoppers, as well as minor pests, were probably controlled by this application although their populations were so low that differences were not measured.

There was little difference between the yield of plants treated with DDT and the check. This spray was applied too late in the season to reduce the infestation of most insects recorded on the plot. Had there been an infestation of corn earworm, as was expected earlier in the season, there would undoubtedly have been a greater difference in yield over the check.

The phorate-Arasan 75 seed treatments produced a slight increase in yield in only one instance. In all others there was a reduction in yield. The poor germination of seed as a result of the phorate-Arasan 75 treatment appeared to be the primary factor responsible for this low yield. The difference in the formulation and the application rate of the phorate and Arasan 75 appeared to have little effect upon the germination of treated seed or the yield of plants from treated seed. Unfortunately, populations of sucking insects were extremely low on plants during the entire growing season. As an unfortunate consequence the value of phorate, as a seed treatment, was not determined. It was also impossible to determine the overall effect of thrips feeding on the yield of soybeans, or the feasibility of applying an insecticide for their control. There was no indication from these results that thrips injury caused a serious enough loss in yields to require control measures.

Table 1.—Yields of whole pods obtained from soybeans grown in experimental plot at Aquasco, Maryland.

Treatment	Yield in Lb./30 Ft. of Row in Each Replication			
	Rep. 1	Rep. 2	Rep. 3	Rep. 4
A (phorate-Arasan)	3.38		2.86	
B (phorate-Arasan)		2.12		3.85
C (malathion)	5.38	5.31	5.67	6.15
D (DDT)	4.84	3.59	4.91	5.36
Check	3.63	3.26	4.71	3.18

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The Designation of Chemicals in Terms of the Responses They Elicit from Insects[1]

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Abstract

The terms "attractant" and "repellent" only have commonly been employed to describe chemicals in terms of their effect on the behavior of insects. The two terms as defined in their strictest sense do not apply to all possible types of reactions of insects to chemicals. There is a genuine need to clarify the use of terminology in this subject. An analysis of the behavioral effects of chemicals on locomotion, feeding, and oviposition has led to the designation and definition of five terms; namely, arrestant, stimulant (locomotor, feeding, ovipositional), attractant, repellent, deterrent. It is proposed that these five be employed as standard terms within the limits of the definitions given.

Chemicals are known to affect the behavior of insects in numerous ways. Despite the diversity of behavior patterns elicited, the terms attractant and repellent only have commonly been employed to describe chemicals in terms of the response patterns. Recently it has been realized that these two terms as defined in their strictest sense do not apply to all possible types of reactions of insects to chemicals. Accordingly, certain authors have been led to coin new terms (Thorsteinson 1953, 1955, Beck 1956, Dethier 1956a), and others, to broaden the definition of the terms "attractant" and "repellent "(Kennedy 1947, Dethier 1947) to make up the deficiency. The purpose of this article is to show some of the difficulties involved when only the terms "attractant" and "repellent" are employed and to discuss some of the suggestions that have been made to overcome the problems.

Attractant.—The term "attractant" has frequently been used to describe substances that cause insects to aggregate, whatever the mechanism. Since true attraction is properly defined as "drawing from a distance," it is obvious that the term "attractant" is often incorrectly applied. One very obvious example is that of substances such as sugar, which have negligible vapor pressure. Sugar itself has almost universally been termed an "attractant." Dethier (1955) went to some length to demonstrate that the action whereby sugar is responsible for an aggregation of insects (*e.g.*, flies) is an orthokinetic reaction. In other words, flies that located a supply of sugar by chance or by nonchemical stimuli (*e.g.*, visual) were induced to stop locomotion and to remain on the sugar by the stimulation of receptors which initiated feeding. No true attraction, that is, drawing from a distance, was in operation. Dethier (1956a) coined the etymologically incorrect word "acceptant" to apply to sugar acting in this manner.

In attempting to resolve the confusion surrounding the use of the word "attractant," Dethier (1956a) pointed out that by definition an attractant causes an insect to perform directive locomotory responses toward a source of stimulation. Endeavoring to elucidate, he added that the definition implies that the material in question acts at some distance from its source. This implication is not a good criterion because in fact the molecules, which actually constitute the stimulus, act at the receptor site just as do sugar molecules. In a further attempt to clarify the situation, behavioral rather than chemical and physical criteria were erected. It was pointed out that since an attractant releases a locomotory response the attractive compound is not a goal in itself but rather releases appetitive behavior. When the source of odor is reached, a new behavior (*e.g.*, feeding, oviposition, mating) is released and this may be a consummative act. An odor which releases such a consummative act is not, strictly speaking, acting as an attractant at the time (Dethier 1956a).

Repellent.—The word "repellent" has likewise frequently been incorrectly used. Two attempts were made to broaden the definition of the term. Dethier (1947) defined a repellent as "any stimulus which elicits an avoiding reaction." He made a further distinction in terms of the physical state of the chemical by recognizing vapor repellents and contact repellents. At the same time Kennedy (1947) pointed out that some reactions to chemicals may taken the form of merely random activity as compared with activity directed away from a source. Reactions may be quick or slow to appear and weak or strong in character. He suggested that the term "repellency" be confined to observed effects on distribution, that it not be used to describe the reactions of insects, that it involves reactions but is not a reaction, and that it is not necessarily of a normal sensory nature. "For practical purposes most workers would agree that a surface is repellent if insects are found to spend less time and so occur in smaller numbers on it than on other available and comparable surfaces." In further discussion of this problem Dethier (1956b) distinguished the two kinds of repellency by describing one as an immediate directional avoiding reaction (taxis) and the other as some sort of irritating effect which causes insects to increase their activity (orthokinesis) with the result that there would be a decreased number on the surface causing excitation.

It is obvious from these brief considerations that the term "repellent" has been applied to chemicals which affect behavior in different ways; furthermore, any one chemical may affect behavior in more than one way. The terms "attractant" and "repellent" have been used to describe chemicals which elicit or prevent feeding and oviposition. This type of use could have arisen from the fact that a compound that has been shown to cause oriented aggregation has subsequently been shown to stimulate insects to feed or oviposit, or that a substance that has been shown to cause avoiding reactions has later been

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[2] Zoological Laboratory, University of Pennsylvania, Philadelphia 4, Pennsylvania: Commonwealth Scientific and Industrial Research Organization, Canberra, Australia; and Entomology Research Division, Agric. Res. Serv., U.S.D.A., respectively.

[3] This manuscript was read by several persons, including Professors Stanley D. Beck, K. D. Roeder, and A. J. Thorsteinson. Their comments and criticism have greatly assisted the authors in clarifying and presenting the concepts presented here. However, the authors accept sole responsibility for proposing the adoption of the terms and concepts presented.

shown to prevent feeding or oviposition. In these cases the interaction between the chemicals and the insect has been defined, not by its nature, but by the designation of the compound which has caused the reaction. An additional complication is introduced by the fact that the same compound may release nonlocomotory reactions at one concentration, true attraction at another, and repellence at still another (*cf.*, *e.g.* Schwinck 1954, Dethier 1954, 1957).

From the foregoing discussion it is obvious that considerable confusion surrounds the terminology in this subject. The problems raised by this confusion are truly of more than academic interest because the direction which future research on attractants and repellents will take is influenced by the degree to which the fundamental nature of the process is understood.

TYPES OF BEHAVIOR ELICITED BY CHEMICALS.—Since the problem under consideration is that of defining chemicals in terms of the behavior that they elicit from insects, the first logical step is an analysis of the types of behavior elicited, after which appropriate terms can be appended. One may describe either the end step in behavior or the mechanism whereby the ultimate behavior was achieved. If a description of only the end step is elected it must be said in the case of locomotory behavior that the insects either aggregate at a spot or disperse from a spot. The terms "attractant" and "repellent" would suffice to designate these conditions, and this is the manner in which these terms are now very loosely employed. The reasons that indicate the undesirability of this system have already been stated. On the other hand, if the mechanisms are envisioned whereby the ultimate step in behavior is achieved, the field of orienting mechanisms is entered. Despite the critical attention of students of behavior (*e.g.* Fraenkel & Gunn 1940, Koehler 1950) to orientation, satisfactory comprehension of events at the neural level awaits further experimental analysis. Even though our knowledge at this level is incomplete, enough is presently known to indicate quite clearly that such behavioral end reactions, as, for example, aggregation, may be brought about in a variety of ways.

The present communication is not intended to supply these desiderata or to constitute a complete analysis or revision of existing schemes of behavior; however, an outline of pertinent modes of locomotory behavior is essential to an analysis of the action of chemicals on locomotion. This is the sole purpose of the scheme given in this paper. It must be stated most emphatically that the scheme is merely a logical structure erected in order to insure that no ambiguities creep into the proposed terminology. The scheme deals exclusively with chemical stimuli. There is full realization that feeding, ovipositional, and locomotory behavior are regulated by other kinds of stimuli which often operate in conjunction with chemical stimuli, but these fall outside the scope of this discussion.

For purposes of this discussion the assumption is made that the insect is either moving or stationary in a chemically inert environment. Under these circumstances a chemical stimulus may elicit certain types of initial behavior for reasons stated in the preceding paragraph. We must be concerned with the first aspect of behavior released.

If an insect is initially moving, chemical stimulation may cause it to react in one or more of the following ways. It may:

(1)	Continue without change of rate of linear progression, rate of turning, or direction.	
(2)	Stop.	
(3)	Slow its rate of linear progression.	Nondirected responses
(4)	Increase its rate of turning.	
(5)	Increase its rate of linear progression.	
(6)	Decrease its rate of turning.	
(7)	Orient toward a source.	Directed responses
(8)	Orient away from a source.	

An insect initially at rest may react to chemical stimuli either by moving or remaining at rest. Once moving, it may react in any of the ways listed above.

Since locomotion is not the only aspect of behavior affected by chemicals, a similar scheme may be erected for feeding, mating, and oviposition. One scheme suffices for both. If an insect is feeding, a chemical may cause it to stop or permit it to continue. If the insect is not feeding, a chemical may or may not initiate feeding.

Having categorized the types of behavior elicited by chemicals, it is now possible to list the types of chemicals in terms of what they do.

Movement:

(1)	Stops or slows	(arrestant)	*e.g.* sugar, +odor
(2)	Starts or speeds	(locomotor stimulant)	*e.g.* DDT, pyrethrum
(3)	Orients toward	(attractant)	*e.g.* +odor (geraniol)
(4)	Orients away	(repellent)	*e.g.* −odor (Indalone)[4]

Feeding, mating, and oviposition stimulant:

(1)	Initiates or drives	(feeding or ovipositional stimulant)	*e.g.* sugar, +odor
(2)	Inhibits	(deterrent)	*e.g.* HCl, demissin, −odor

To designate these actions the following terms are defined:

(1) *Arrestant*—a chemical which causes insects to aggregate in contact with it, the mechanism of aggregation being kinetic or having a kinetic component. An arrestant may slow the linear progression of the insects by reducing actual speed of locomotion or by increasing turning rate. (It is believed that the creation of the *-ant* form of this word is etymologically correct since *arrest* is derived through Old French presumably from the Vulgar Latin *arrestare* [Latin *ad*+*restare*] and may thus properly take this suffix.)

(2) *Locomotor stimulant*—a chemical which causes, by a kinetic mechanism, insects to disperse from a region more rapidly than if the area did not contain the chemical. The effect may be to increase the speed of locomotion, to cause the insects to carry out avoiding reactions, or to decrease the rate of turning (Fraenkel & Gunn 1940).

(3) *Attractant*—a chemical which causes insects to make oriented movements towards its source.

(4) *Repellent*—a chemical which causes insects to make oriented movements away from its source.

(5) *Feeding, mating, or ovipositional stimulant*—a chemical which elicits feeding or oviposition in insects. The term "feeding stimulant" is synonymous with "phagostimulant" coined by Thorsteinson (1953, 1955). Beck

[4] Butyl 3,4-dihydro-2,2-dimethyl-4-oxo-2*H*-pyran-6-carboxylate.

(1956) employed the term "saccharotrophism" in the same context to refer to the behavior of an insect stimulated by sugar to feed.

(6) *Deterrent*—a chemical which inhibits feeding or oviposition when present in a place where insects would, in its absence, feed or oviposit. This term has been used in the intended sense in referring to *rodent deterrents*.

It is possible to make several generalizations about the use of these terms: (a) the same compound may have multiple effects on behavior; (b) any given effect may be elicited by chemicals which in other respects act differently; (c) movements involving orientation can be evoked by concentration gradients or by currents carrying a chemical which may not necessarily have to be present as a gradient; there is also a theoretical possibility that solids dispersed in a gradient of density may elicit oriented responses.

At the present time there appears to be a genuine need for the terms "attractant," "repellent," "arrestant," "stimulant," and "deterrent." It is proposed that these five be employed as standard terms within the limits of the definitions given above. Furthermore, the terms should always be employed when designating specific cases. In other words, if a given amino acid is an attractant under one set of circumstances and elicits feeding or oviposition under another set of conditions, the statement should not be made that this attractant elicits feeding and oviposition. To do this is to defeat the fundamental purpose of a useful terminology designed to describe chemicals with respect to their effects on behavior.

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Persistence of DDT, Aldrin, and Lindane in Some Midwestern Soils[1,2]

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ABSTRACT

Seven fields (Ulysses silt loam, Kansas; Knox silt loam, Kansas; muck, Ohio; sandy loam, Ohio; sandy loam, Miami silt loam, and muck, Wisconsin) were treated in 1954 with DDT (10, 100 and 1,000 lb./6″ acre), aldrin (2, 20 and 200 lb./6″ acre) and lindane (1, 10, and 100 lb./6″ acre). DDT was found to be the most persistent of the three. Lindane had the fastest dissipation rate. Soils treated with aldrin at 2 lb./6″ acre in 1954 contained no aldrin in the fall of 1958, but 0.03 to 0.19 p.p.m. of dieldrin was recovered. No lindane was detected in soils 4½ years after treatment at 1 lb./6″ acre. All three insecticides disappeared most rapidly in the Kansas experimental soils. Two major factors affecting the persistence of an insecticide in soils appear to be the amount of organic matter within a particular soil type and the climatic conditions of the area.

The persistence of insecticides in soils and their possible accumulation following repeated application has been extensively studied (Boswell *et al.* 1955, Chisholm *et al.* 1951, Fleming *et al.* 1951, Ginsburg 1955, Lichtenstein 1957, Lichtenstein & Schulz 1959b, and Lichtenstein & Polivka 1959). In addition it has been shown that after the application of a certain insecticide to soil, breakdown products of the insecticides may appear, which still may be toxic to insects or even more toxic than the parent compound (Edwards *et al.* 1957, Gannon & Bigger 1958, Lichtenstein & Schulz 1959a). Pesticide residues in soils may affect crop growth, soil microflora and microfauna. In addition they may be absorbed by plants and translocated into crop tissues. In order to study the

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mechanism of insecticide loss from soils seven field plots in three Midwestern States were established in 1954. DDT, aldrin, and lindane were applied to various soil types, to study the effect of soil type, climatic conditions, and the rate of application on their persistence.

EXPERIMENTAL FIELDS AND THEIR LOCATIONS.—The seven experimental fields established in the spring of 1954 were: two silt loam soils in Kansas (each 1½ acre), one muck and one sandy loam in Ohio (each ⅛ acre), and a muck, a silt loam, and a sandy loam in Wisconsin (each 1½ acre). Though three similar soil types (muck, silt loam, and sandy loam) were used, all differed greatly in many properties, such as organic matter content and water-holding capacity (table 1).

The experimental field in Garden City, Kansas, is an Ulysses silt loam, on which no crops had been grown previously, nor had any insecticide been previously applied. The other Kansas field is located near Wathena, and the soil type is classified as a Knox silt loam. Until 1946 the field was in apple orchard, and later was planted with alfalfa, corn, and wheat. Lead arsenate had been applied to the orchard, bnt no insecticides had been used since 1945.

In Ohio the muck plot is located near Celeryville and the sandy loam near Marietta. Potatoes were grown for some years on both fields and DDT had been used several times. However, no records were kept as to the spray schedules.

In Wisconsin all three experimental fields (muck, Miami silt loam, and Fox fine sandy loam) are located in the immediate vicinity of Madison. No insecticides had been used before the start of the experiment.

Climatological data for the areas are summarized in table 2.

METHODS.—Each experimental field was subdivided into 12 plots (10′×30′ in Ohio, 29′×100′ in Kansas and Wisconsin) separated by buffer areas (5′ wide in Ohio, 15′ wide in Kansas and Wisconsin). Three of the randomized 12 plots were used as untreated controls, and the remaining 9 were treated with 3 insecticides at 3 different dosages. DDT (25% emulsion—2 lb./gal.) was applied at the rates of 10, 100, and 1,000 lb./acre as technical material, aldrin (21.7% emulsion—2 lb./gal.) at the rates of 2, 20, and 200 lb./acre and lindane (20% emulsion—1.62 lb./gal.) at the rates of 1, 10, and 100 lb./acre. Except for DDT at a rate of 1,000 lb./acre, which was applied as an undiluted emulsion concentrate, all the insecticidal applications involved a thorough mixing of a measured amount of emulsifiable concentrate with 20 gallons of water. The area to be treated was was divided into ten 10′×29′ subplots and 2-gallon quantities of the diluted emulsion were spread with a sprinkling can as uniformly as possible over each subplot. In Ohio, because of the smaller plot size, the necessary amount of emulsion concentrate was diluted with 2 gallons of water, and no subplots had to be established. The plots were treated in May 1954 in Kansas and Wisconsin, and in June 1954 in Ohio. After treatment, each plot was rototilled (Ohio, Wisconsin) or disced (Kansas) to a depth of 4 to 6 inches. Later on a rototiller or disc was used to keep in fallow those parts of the plots that were used for soil sampling.

Soils were sampled initially and at 0.5, 1.5, 2.5, 3.5, and 4.5 years thereafter. The fallow areas sampled in Kansas and Wisconsin measured 29′×50′. In Ohio the whole plot was used. To avoid contamination by water movement, cultivation, and other factors, the actual area sampled was reduced to 21′×42′ (Kansas and Wisconsin), leaving a 4′ strip between the boundaries of the treated plot and the sampling area. This strip amounted to a width of only 1′ on the Ohio plots. A standard soil sampling auger (fig. 1) with a sleeve attached made it possible to secure full-sized cores (¾″ diameter, 6″ deep) while pulling the tube out of the soil. A total of 40 cores

Table 2.—Climatological data for field plot locations, 1954–58.

LOCATION	TOTAL PRECIPITATION (INCH)	AVERAGE TEMPERATURES (° F.)
Wathena—Kansas	159.44	54.2±1.23
Garden City—Kansas	87.05	54.1±1.25
Marietta—Ohio	212.76	54.1±1.55
Celeryville—Ohio	185.82	50.6±1.44
Madison—Wisconsin	143.02	46.9±0.77

Table 1.—Some soil properties of the seven insecticide-treated fields.

LOCATION AND SOIL TYPE	DRY WEIGHT IN POUNDS/1A., 6″ DEEP	WATER HOLDING CAPACITY (%)	ORGANIC MATTER (%)	pH	POUNDS/6⅔″ ACRE		
					N	P	K
Kansas							
1) Garden City Ulysses silt loam	1,762,000	65	1.8	6.9	175	400	800
2) Wathena Knox silt loam	1,860,000	70	0.8	6.8	175	135	300
Ohio							
3) Celeryville Muck	562,000	150	74.5	4.9	600	72	150
4) Marietta Sandy loam	1,793,000	42	2.0	6.0	175	300	430
Wisconsin (Madison)							
5) Fox fine sandy loam	1,895,000	30	0.8	7.2	175	145	102
6) Miami silt loam	1,610,000	60	3.6	7.1	250	140	170
7) Muck	665,000	130	40.0	6.8	600	700	180

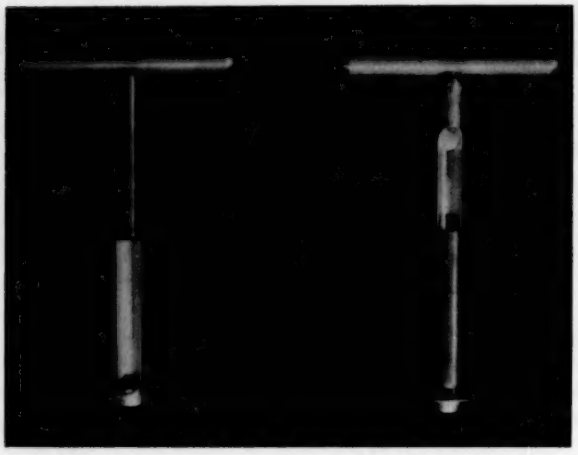

FIG. 1.—Soil sampling auger with attached sleeve for securing full sized cores.

collected from each plot and placed in plastic bags within an ice cream carton was shipped to Wisconsin, where the samples were analyzed.

On the basis of the oven-dry weight of those 40 cores, the dry weight of 1 acre, 6″ deep, could be calculated (table 1). This allowed expression of the application rates of the insecticides on a part per million basis. As shown in table 3, one pound of insecticide per 6″-acre amounts to 0.53–0.61 p.p.m. in loamy soils and to 1.5–1.8 p.p.m. in muck soils.

ANALYTICAL METHODS.—*Colorimetric Analyses.*—The samples were handled and extracted as described by Lichtenstein & Schulz (1959a). The extraction solvents used were a mixture of benzene-isopropanol (2:1) for DDT and a mixture of redistilled hexane-isopropanol (2:1) for aldrin and dieldrin.

DDT extracts were cleaned up by shaking with activated carbon or by passing through a column (6″×1″) of aluminum oxide and analyzed according to the Schechter-Haller method (1945). Known amounts of DDT added to soils were recovered to an extent of 92% to 98%.

When soils treated with aldrin were analyzed for aldrin only, cleanup was accomplished by passing the extract through a column (8″×1″) of Attasol-Celite (3:2). However, when the extracts were analyzed for aldrin and dieldrin, cleanup as well as separation of the two insecticides was done by using a column (8″×1″) of magnesium oxide-Celite (2:1), which had been heated for 24 hours at a temperature of 130° C. After the extract containing aldrin and dieldrin had been added to the column, aldrin was eluted by adding 75 ml. of redistilled hexane. Dieldrin could then be washed from the column with an additional 600 ml. of the same solvent. Aldrin and dieldrin fractions were analyzed according to the method of O'Donnell *et al.* (1954, 1955). Known amounts of aldrin added to soils were recovered to an extent of 92% to 97% and known amounts of dieldrin added to soils were recovered to an extent of 90% to 94%. Lindane was analyzed according to the Schechter-Hornstein method (1952). A change of the method (Lichtenstein *et al.* 1956) eliminated a special extraction procedure and permitted the determination of lindane directly in soils and crops. Known amounts of lindane added to soils were recovered to nearly 100%.

Each analysis was run in duplicate using a soil blank for the determination of apparent insecticide. In addition known amounts of insecticide were added to insecticide-free soil samples. This method permitted a check of the analytical procedure for each analysis done. The unknowns after the values for apparent insecticide had been subtracted were calculated on the basis of the values obtained for the known amounts. Results were expressed in parts per million based on the dry weight of the soil under investigation.

Bioassays.—*Drosophila melanogaster* Meig. was used as the test insect. In most cases a direct feeding method was employed (Edwards *et al.* 1957) where the flies were exposed to the soils directly, thus eliminating a special extraction procedure. However, when soils containing aldrin and dieldrin were tested, bioassays were made both by the direct exposure method and by exposing flies to dry films of extracts containing either aldrin or dieldrin.

RESULTS AND DISCUSSION.—The recoveries of DDT, aldrin, and lindane from all seven soil types during a period of 4½ years after soil application are summarized in table 4. The result from specific colorimetric analyses for all three insecticides indicated that DDT was most persistent and aldrin least persistent. The insecticidal persistence was greater in muck soils than in loamy soils. The loam plots located in Ohio and Wisconsin retained the insecticides to a larger extent than those located in Kansas.

The most rapid decline of the insecticidal residues occurred during the first half year after soil treatment. The change in the rate of loss of the insecticides with time was most pronounced for aldrin and lindane and least pronounced for DDT, as was previously noted (Lichtenstein & Schulz 1959b).

DDT.—The amounts recovered 4½ years after soil treatment (autumn of 1958) ranged from 23% to 62% of the applied dosage in Wisconsin to 33% in Ohio (see footnote 3, table 4) and from 4% to 28% in Kansas. Only 4% to 7% of the applied dosage was recovered in Wathena, Kansas.

Lindane was recovered in the fall of 1958 to an extent of 0 to 36% of the applied dosage from the muck soils and

Table 3.—Application rates in p.p.m., based on the dry weight of 1 acre, 6″ deep.

LOCATION AND SOIL TYPE	APPLIED (LB./ACRE)[a]		
	DDT (technical) 10	Aldrin 2	Lindane 1
Kansas			
Ulysses silt loam	4.76[b]	1.13	0.56
Knox silt loam	4.51	1.07	0.53
Ohio			
Muck	15.0	3.56	1.78
Sandy loam	4.82	1.15	0.58
Wisconsin			
Fox fine sandy loam	4.42	1.05	0.53
Miami silt loam	5.25	1.25	0.61
Muck	12.75	3.02	1.51

[a] Ten and 100 times application rates would result in 10 and 100 times p.p.m. values.

[b] For all DDT data: p,p′-isomer = 84% of technical DDT.

Table 4.—Recoveries of DDT, aldrin, and lindane from seven field plots (colorimetric analyses).

Location and Soil Type	Applied (Lb./6″-Acre) Spring 1954	Recovered in Per Cent of Calculated Application Fall 1954	1955	1956	1957	1958
		DDT(p'p isomer)				
Kansas						
Ulysses silt loam	8.4	72.5	26.0	22.0	19.6	19.0
	84.0	56.0	29.0	22.0	17.8	17.8
	840.0	67.5	35.0	18.3	25.2	27.8
Knox silt loam	8.4[a]	157.0	114.0	81.5	52.2	73.0
	84.0	86.0	15.0	5.8	9.0	6.8
	840.0	92.0	11.4	11.8	13.6[b]	4.0
Ohio						
Muck	8.4	66.0	22.5	15.3	[c]	23.0
	84.0	59.5	41.5	29.4	[c]	26.4
	840.0	57.0	53.0	30.2	[c]	37.7
Sandy loam	8.4[d]	222.0	650.0	592.0	675.0	635.0
	84.0	85.0	65.5	59.0	33.0	32.8
	840.0[d]	83.1	43.5	40.2	31.8	32.7
Wisconsin						
Fox fine sandy loam	8.4	84.0	55.0	37.3	34.7	39.0
	84.0	89.0	61.2	52.1	42.0	46.0
	840.0	94.0	70.5	59.2	53.8	62.0
Miami silt loam	8.4	67.5[e]	41.6[e]	28.5[e]	22.6[e]	30.4[f]
	84.0	90.0	68.5	56.5	46.0	50.5
	840.0	94.0	81.0	61.2	57.5	60.8
Muck	8.4	81.5	60.0	43.8	32.4	23.0
	84.0	117.0	92.0	50.5	44.0	28.4
	840.0	73.0	64.0	61.0	52.0	30.0
		Aldrin				
Kansas						
Ulysses silt loam	2	21.0	0.0	0.0	0.0	0.0
	20	25.2	4.5	1.8	0.3	0.0
	200	55.0	12.1	7.5	3.7	1.3
Knox silt loam	2	5.5	0.0	0.0	0.0	0.0
	20	29.8	0.2	0.6	0.7	0.0
	200	22.0	1.4	0.3	0.2	0.0
Ohio						
Muck	2	42.2	18.3	3.7	11.3	8.1
	20	63.5	40.6	28.3	49.2[e]	17.5
	200	72.0	50.5	40.0	38.4	25.7
Sandy loam	2	4.6	3.5	0.0	0.0	0.0
	20	31.8	9.3	4.4	1.4	1.1
	200	28.0	8.5	3.3	0.7	0.3
Wisconsin						
Fox fine sandy loam	2	2.5	0.0	0.0	0.0	0.0
	20	9.2	2.7	0.8	0.7	0.4
	200	36.0	23.3	11.5	5.4	2.0
Miami silt loam	2	4.0	0.0	0.0	0.0	0.0
	20	17.6	8.3	3.0	1.1	0.8
	200	26.0[e]	13.1[e]	5.7[e]	3.0[e]	3.9[f]
Muck	2	26.5	10.3	6.3	2.3	0.0
	20	34.2	16.4	10.3	4.7	2.5
	200	73.5	52.5	33.0	21.0	12.5
		Lindane				
Kansas						
Ulysses silt loam	1	56.0	4.9	0.0	0.0	0.0
	10	49.0	17.0	13.2	6.5	3.7
	100	55.0	28.8	16.4	10.0	7.2
Knox silt loam	1	21.8	0.0	0.0	0.0	0.0
	10	29.1	4.8	1.1	0.4	0.0
	100	49.8	3.3	2.1	0.4	0.3
Ohio						
Muck	1	80.0	47.0	13.5	9.0	0.0
	20	81.5	62.5	41.0	29.7	27.8
	100	77.0	62.0	63.5	33.0	36.2
Sandy loam	1	17.4	0.0	0.0	0.0	0.0
	10	52.2	32.0	15.0	10.3	5.2
	100	62.5	45.7	20.0	15.0	11.0
Wisconsin						
Fox fine sandy loam	1	22.6	0.0	0.0	0.0	0.0
	10	58.0	34.5	18.3	12.0	6.9
	100	62.0	48.5	33.5	23.0	17.8
Miami silt loam	1	19.6	14.7	0.0	0.0	0.0
	10	43.2[e]	24.2[e]	9.5[e]	3.9[e]	5.7[f]
	100	55.1	36.1	19.2	13.7	11.2
Muck	1	15.9	6.6	11.8	10.3	0.0
	10	79.0	58.0	40.2	33.0	23.6
	100	85.0	76.0	52.5	48.5	36.7

0 to 18% from the loam soils. However, only 0 to 7% of the applied dosage was recovered in Garden City, Kansas, and only traces of lindane were found in the Wathena, Kansas, plot treated at a rate of 100 lb./6″ acre.

Aldrin, as assayed by a specific colorimetric method, is the least persistent of the three insecticides. It disappeared fastest in the loam plot located at Wathena, Kansas, where one-half year after soil treatment only 6% to 30% of the applied dosage was recovered from the soil. Most of the insecticide had disappeared 1 year later. In the fall of 1958 no aldrin was found in any of the Wathena, Kansas, plots, However, all the other loam plots contained aldrin at that time to an extent of 0 to 4% of the applied dosages. More aldrin was found in the muck soils (0 to 26% of the applied dosages).

Figure 2 illustrates the rate of loss of aldrin residues from plots having been treated at 20 lb./6″ acre. It seems that the most important factors responsible for the persistence of insecticides in soils are the amount of organic matter within a particular soil type and the climatic conditions of the area. Moreover, in nearly all cases investigated, the insecticides persisted longest in those soils treated at the highest application rate.

When bioassay procedures were employed for analyzing aldrin-treated soils, recovery data obtained by bioassay were usually higher than those obtained by chemical analyses. This fact seemed to indicate that another toxicant had appeared which was not detected by the specific colorimetric analysis for aldrin. This toxicant was found to be dieldrin (Lichtenstein & Schulz 1959a). However, when flies were exposed to a dry film of extracts (aldrin or dieldrin fractions) bioassay results were in agreement with those obtained by chemical analyses. Many soils sampled in the fall of 1958 did not contain any aldrin. Yet, flies exposed to those soils revealed the presence of another toxicant, which in all cases was found to be dieldrin. The residues of aldrin and dieldrin recovered 4½ years after soil treatment with aldrin are summarized in table 5. With the exception of the muck soil in Ohio, no aldrin was found in any soil that had been treated at a rate of 2 lb./6″ acre. Yet, 0.03 to 0.16 p.p.m. of dieldrin were recovered.

It was found that the conversion of aldrin to dieldrin was least in muck soils and most in sandy loams where 88% to 96% of the total toxicants were recovered as dieldrin. Again the Kansas plots were outstanding by containing the smallest amount of dieldrin (0 to 2.6% of the amount of aldrin applied 4½ years previously). The largest amounts of aldrin and dieldrin were present in the Ohio muck soil and the least in the two Kansas loam soils.

Lindane has been reported (Lichtenstein & Schulz 1959a) to break down in soils to a noninsecticidal compound which still responds to the Schechter & Hornstein

[a] Initial analysis resulted in a recovery of 169% of the calculated application rate.

[b] Soil sample was extremely muddy.

[c] Entire area had been flooded several times. Checks were contaminated with DDT.

[d] During flash rains, soil was washed yearly from the 1,000-lb./acre-treated-DDT plot onto the 1 treated at a rate of 10 lb./acre (8.4 lb. p,p'-isomer.)

[e] Upper section of slightly sloped plot.

[f] Middle section of slightly sloped plot. Upper section was lost in 1958 because of road construction.

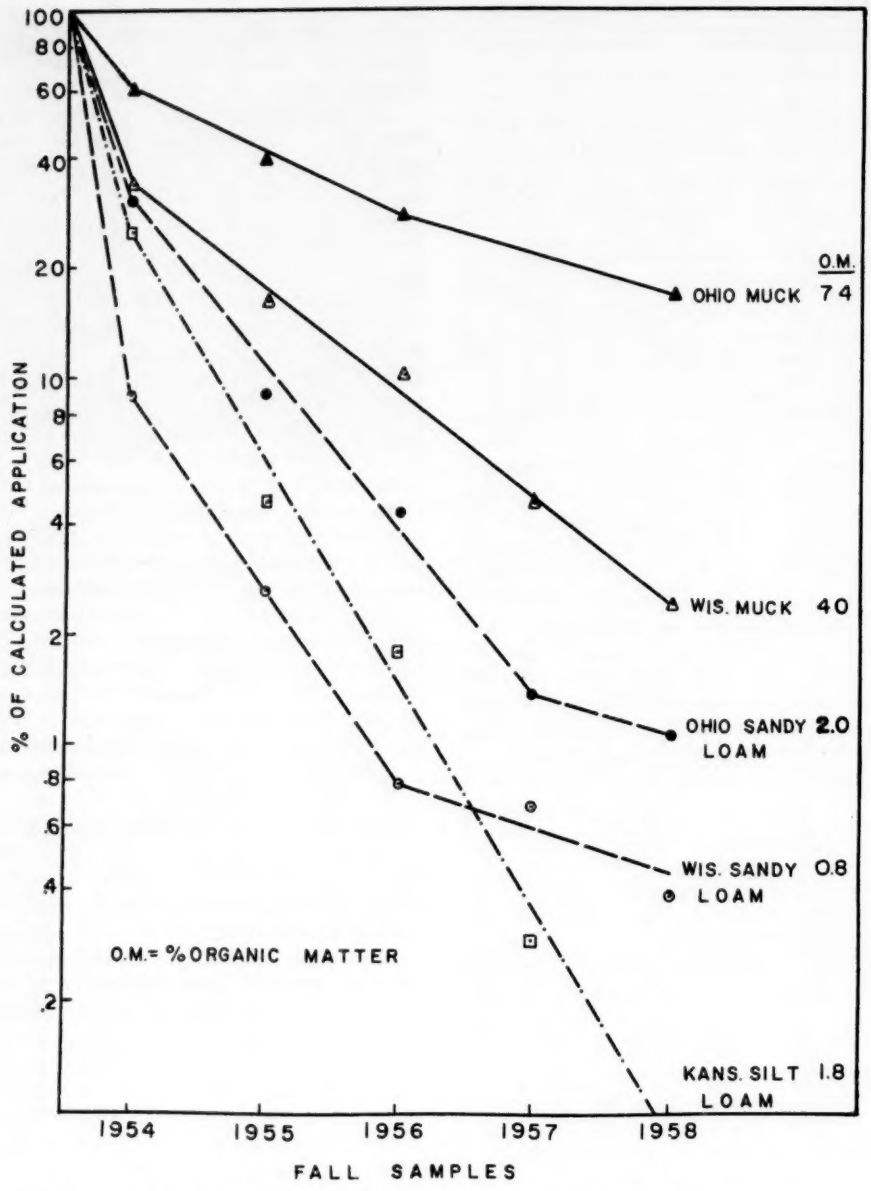

FIG. 2.—Rate of loss of aldrin residues from various soils. Treatment: 20 lb./6″-acre.

method (1952) for analyzing lindane. Therefore, data obtained by bioassay procedures, testing residual toxicity, seem to offer a more realistic assessment of toxic residues present owing to soil treatment with lindane. Whereas residue data from colorimetric analyses presented in table 4 for aldrin are actually too low, those presented for lindane are too high. Table 6 summarizes the residues found in the lindane-treated soils as determined by chemical analyses and bioassays, 4½ years after soil treatment. The recoveries obtained by bioassays amounted to 26% to 78% of the estimates obtained by chemical analyses. Only in the muck plot treated in Ohio with lindane at a rate of 1 lb./acre and in the sandy loam (Ohio) treated at a rate of 10 lb./acre, were amounts recorded by bioassay greater than those obtained by chemical analyses. This condition might be explained by the fact that those plots has been flooded several times, which possibly resulted in contamination of one plot by another.

With the exception of the Ohio-muck, all the other experimental fields treated in 1954 with lindane at a rate of 1 lb./6″ acre did not contain any toxicants when bioassayed in the fall of 1958. Even those plots, treated with lindane at a rate of 10 lb./6″ acre in the Kansas experimental fields, did not contain any toxicants, whereas the loam plots in Ohio and Wisconsin still contained 0.09 to 0.35 p.p.m. and the muck plots 2.63 and 2.45 p.p.m. When the plots treated with lindane at a rate of 100 lb./6″ acre were examined, it was found that in Kansas only 0.04 p.p.m. (Wathena) and 1.32 p.p.m. (Garden City) were present. Comparably treated soils in Ohio and Wisconsin contained considerably more toxicants and the amounts recovered by bioassay on both locations and in

Table 5.—Aldrin and dieldrin residues from seven soil types, 4½ years after treatment with aldrin.[a]

Location and Soil Type	Applied (Lb./6″-Acre)								
	2			20			200		
	Recovered in p.p.m.								
	Total[b]	Per Cent of Total		Total	Per Cent of Total		Total	Per Cent of Total	
	A and D	A	D	A and D	A	D	A and D	A	D
Ohio									
Muck	0.48	61.0	39.0	11.7	53.5	46.5	131	70.0	30.0
Wisconsin									
Muck	0.16	0.00	100	2.77	27.0	73.0	63.1	60.0	40.0
Miami silt loam	0.03	0.00	100	0.36	27.5	72.5	11.3	44.2	55.8
Kansas									
Ulysses silt loam	0.00	0.00	0.00	0.16	0.00	100	4.5	32.7	67.3
Knox silt loam	0.00	0.00	0.00	0.17	0.00	100	0.27	0.00	100
Ohio									
Sandy loam	0.10	0.00	100	3.11	4.2	95.8	7.13	5.5	94.5
Wisconsin									
Sandy loam	0.06	0.00	100	0.97	4.1	95.9	16.5	12.5	87.5

[a] Chemical analyses.
[b] A, aldrin; D, dieldrin.

similar soil types were nearly identical.

All three insecticides were least persistent in the Kansas experimental plots, where the most rapid decline was noticed at Wathena. Though in both Kansas locations temperatures were equal during the 5-year period (1954 to 1958), total precipitation in both locations was considerably different (table 2). A total of 159.44 inches of precipitation was recorded during those 5 years at Wathena and only 87.05 inches at Garden City. Since insecticides are more persistent in dry soils than in wet soils (unpublished data), this condition might explain to some extent the relatively longer persistence of the insecticides in the Garden City Ulysses silt loam. Moreover, this soil has an organic matter content of 1.8%, whereas only 0.8% of organic matter was found within the Wathena Knox silt loam. In addition, the Kansas plots, particularly Garden City, were subjected to high wind erosion.

To compare the persistence of DDT, aldrin, and lindane, some recovery data of these insecticides, 4½ years after treatment of a Miami silt loam, have been summarized in table 7. By using specific colorimetric methods, as well as a bioassay procedure, more interpretable information was obtained on the actual amount of toxicants present in the soil. DDT was the most persistent insecticide of the three tested, followed by aldrin. Because of the conversion of aldrin to dieldrin in soils and the longer residual properties of dieldrin, the sum of aldrin and dieldrin present has to be taken into consideration, though these two insecticides may not be equitoxic on any particular insect. Lindane was the least persistent of the three insecticides.

Table 6.—Lindane residues from seven soil types 4½ years after soil treatment.

Location and Soil Type	Applied (Lb./6″-Acre[a])					
	10			100		
	Recovered in Per Cent of Applied					
	Chemical Assay	Bioassay	B/C	Chemical Assay	Bioassay	B/C
Muck (Ohio)	27.8	14.8	53	36.2	28.7	78
Muck (Wisconsin)	23.6	16.1	68	36.7	28.0	76
Miami silt loam (Wisconsin)	5.7	1.5	26	11.2	7.2	63
Ulysses silt loam (Kansas)	3.7	0.0		7.2	2.3	33
Knox silt loam (Kansas)	0.0	0.0		0.25	0.1	30
Sandy loam (Ohio)	5.2	6.1	116	11.0	4.7	42
Sandy loam (Wisconsin)	6.9	2.5	36	17.8	7.0	40

[a] Analyses of the plots treated at a rate of 1 lb./6″ acre yielded 0.00 p.p.m. as tested by chemical and bioassay methods, with the single exception of 0.20 p.p.m. recovered from the Ohio muck as determined by bioassay.

[b] $\frac{B}{C} = \text{Ratio} \frac{\text{Bioassay}}{\text{Chem. assay}} \times 100.$

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Table 7.—Recoveries of DDT, aldrin, dieldrin, and lindane from a Miami silt loam 4½ years after soil treatment.

Applied (Lb./6″-Acre)		Recovered in Per Cent of Applied	
		DDT[a]	*DDT*[b]
DDT	10	30	28
	100	51	54
		Aldrin[a]+*Dieldrin*	*Aldrin*[a]
Aldrin	20	3.5	0.8
	200	10.0	3.9
		Lindane[b]	*Lindane*[a]
Lindane	10	1.5	5.7
	100	7.2	11.2

[a] Chemical analyses.
[b] Bioassay.

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Influence of Plant Age on the Survival of Alfalfa Varieties[1] Differing in Resistance to the Spotted Alfalfa Aphid

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ABSTRACT

The reaction of four alfalfa varieties—Lahontan, Moapa, African, and Caliverde—of three ages of the plants was examined during a prolonged, severe, induced attack of the spotted alfalfa aphid (*Therioaphis maculata* (Buckton)). Increased plant age significantly decreased mortality of resistant varieties Lahontan and Moapa, but had no effect on that of Caliverde except at the shorter exposure period of 9 days. Older plants of African, a more tolerant but susceptible variety, survived slightly longer than the younger seedlings. About half the seedlings of Lahontan and Moapa infested in the cotyledon stage and about one-fourth of those in the second or third trifoliolate leaf stage were killed after 21 days' exposure to the increasing aphid population. Very low mortalities (0% and 3%) were recorded for plants 41 days old when infested. African at all age groups was more tolerant than Caliverde, which was killed in 21 days. The higher survival of Lahontan and Moapa, despite high aphid populations, indicated that tolerance as well as antibiosis contributed to their over-all resistance.

Observations of injury to alfalfa by the spotted alfalfa aphid (*Therioaphis maculata* (Buckton)) have shown a varied but generally high susceptibility of seedlings to natural and induced infestations (Dickson *et al.* 1955, Reynolds *et al.* 1957, Hackerott *et al.* 1958, Nielson 1958). Under severe infestations seedling mortality ranged from 100% for susceptible varieties to 50% or occasionally more for resistant varieties such as Lahontan. In contrast, the present authors found that the mortality of mature plants of susceptible varieties was nearly 100% compared with usually less than 5% for resistant varieties. Knowledge of the influence of plant age on resistance to this aphid is needed to define the hazards of stand establishment and maintenance among varieties differing in resistance. In 1957, tests were made at Bakersfield, California in which plant mortality patterns were simultaneously examined in two resistant and two susceptible varieties in three age groups during a period of epidemic aphid buildup.

METHODS.—Lahontan, Moapa, African, and Caliverde varieties were used, since they had previously demonstrated a wide range in susceptibility and resistance to the spotted alfalfa aphid. Lahontan served as a standard resistant check. Moapa, bred from African selections, is a rapid-growing, aphid-resistant variety adapted to the area. Its inclusion permitted a comparison of its resistance with that in Lahontan and the genetic improvement of resistance over its parents. African, a susceptible variety, was included because it has frequently displayed tolerance by supporting large populations of aphids in the field and nursery with less severe plant mortality than Caliverde. Caliverde is a susceptible variety normally recommended for the area.

Each variety was planted on three dates—May 22, June 18, and July 1—in single 5½-foot rows 8 inches apart at the rate of 1 gram of seed per row. The 12 treatments were completely randomized in 4 replicated 6×10-foot blocks in the nursery. A border row of Caliverde was planted at the ends of each block on the first planting date. Blocks were placed in linear arrangement 10 feet apart in light sandy loam soil. Breeders' seed of Moapa and certified seed of the other varieties were used. Sprin-

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Table 1.—Effect of plant age on spotted alfalfa aphid populations on alfalfa varieties after 8 days' exposure.

Variety	Age of Plants (Days)		
	3	14	41
Lahontan	8	57	213
Moapa	4	96	64
African	10	155	449
Caliverde	10	206	220

kler irrigation was used during the period of stand establishment. During the test period the plots were carefully irrigated without permitting water to accumulate on the soil surface.

Aphids and other insect pests were controlled with spray applications of TEPP as needed until July 8, after which the infestation was allowed to develop. The plant ages of the three groups on this date, based on 50% seedling emergence with expanded cotyledons, were: 3 days with unifoliolate leaves, 14 days with two to three trifoliolate leaves, and 41 days mature plants with succulent regrowth 4 to 6 inches high. Fortunately, heavy aphid populations on plots a few feet away provided conditions for natural infestation by winged adults. On July 11 portable cages (Howe & Smith 1957) were placed over the blocks to exclude predators. Additional aphids were then introduced by shaking equal numbers of heavily infested stems over each block planting. Each caged block was periodically examined and any lady beetles present were killed. The number of plants per row before infestation ranged generally from 35 to 50.

The first population counts were made on July 17, on five plants chosen at random. Severe plant injury began to appear by this time. Owing to the tremendous variation in plant size, a different method of estimating populations was employed for each age group. Counts were made during the cool of the morning when aphids were least active. On the 3-day-old seedlings the aphids were counted on unifoliolate leaflets. A dental mirror was used to examine the under side of the leaflets to avoid disturbing the aphids. Populations on the 14-day single-stemmed seedlings were based on the entire plant. The 41-day-old plants had developed crowns with several stems. It was therefore possible to clip a stem for actual counts without appreciably weakening the plant. Maximum daytime temperatures during the period of aphid buildup and plant injury averaged about 95° F.

Observations of plant mortality were made on July 17, 22, and 29 after exposure periods of 9, 14, and 21 days. Plants were considered dead when all or nearly all the green had disappeared.

RESULTS.—Aphid populations on caged plants increased more rapidly than has been normally observed in the field. In counts made 9 days after exposure to aphids, large populations were found on all varieties (table 1). It was thus apparent that any improved survival of resistant plants would be partially owing to tolerance in addition to the antibiosis mechanism observed in resistant varieties such as Lahontan (Howe & Smith 1957). This pattern of infestation was different from that on adjacent uncaged plots, where resistant varieties carried fewer aphids than susceptible or tolerant varieties (Howe & Pesho 1960). This difference may have been caused by the abnormal conditions owing to the cage, proximity of rows to each other, or the greater intensity of the infestation. As populations developed and injury increased, the reduced growth on the oldest Caliverde plants resulted in a corresponding reduction in its aphid-carrying capacity.

Table 2 records the different trends of plant mortality as the exposure period increased. The multiple-range test (Duncan 1955) was used to determine significance. As expected, Caliverde plants were killed more rapidly than the other three varieties. The 41-day-old plants appeared significantly more resistant only at the 9-day exposure period. Increased age had little effect on plant survival when exposed 14 or more days to epidemic buildup. All age groups of African usually showed significantly lower mortalities than Caliverde at all exposure periods. The mortalities of 3- and 14-day old African seedlings were not significantly different at any exposure period. It is probable that the greater vigor in the 14-day-old plants was offset by their larger population.

The resistant varieties Lahontan and Moapa had similar mortalities in all age groups. Moapa produced greater growth than Lahontan. Both showed a distinctly different mortality pattern from Caliverde and African. The 41-day-old plants of both varieties were nearly im-

Table 2.—Plant mortality of four alfalfa varieties in three age groups after various periods of exposure to the spotted alfalfa aphid—1957.

Exposed 9 Days			Exposed 14 Days			Exposed 21 Days		
Variety	Age (Days)	Mortality[a] (%)	Variety	Age (Days)	Mortality (%)	Variety	Age (Days)	Mortality (%)
Lahontan	41	0	Lahontan	41	0	Lahontan	41	0
Moapa	41	0	Moapa	41	1.5	Moapa	41	3.0
African	41	0	Moapa	14	11.4	Lahontan	14	26.9
Moapa	3	1.4	Lahontan	14	24.2	Moapa	14	27.4
Moapa	14	3.7	Moapa	3	28.6	Lahontan	3	48.9
Lahontan	14	3.8	Lahontan	3	40.2	Moapa	3	52.8
Lahontan	3	5.0	African	41	48.4	African	41	64.4
Caliverde	41	5.5	African	3	63.1	African	14	75.6
African	14	11.3	African	14	64.6	African	3	85.2
African	3	15.5	Caliverde	41	93.7	Caliverde	41	100.0
Caliverde	14	33.2	Caliverde	14	96.2	Caliverde	14	100.0
Caliverde	3	37.9	Caliverde	3	96.6	Caliverde	3	100.0

[a] Means opposite continuous line not significantly different at 5% level.

Table 3.—Mean squares from analyses of variance of the mortality data for plants of different ages.

Source of Variance	Age of Plants (Days)		
	7	14	21
Treatment	715.43*	5,117.48**	5,379.75**
Variety	454.75**	16,939.41**	16,185.80**
Age	1,024.65 ns	1,849.92**	3,570.17**
Variety×age	242.79 ns	295.71 ns	579.46*

* Significant at 5% level.
** Significant at 1% level.

mune to kill, with no more than 3% mortality observed after 21 days. Both seedling groups were more susceptible to kill than the mature plants at increased exposure. After 21 days' exposure the 14-day-old plants showed about half the mortality observed in the 3-day-old seedlings. Both seedling ages of these varieties were usually more resistant than all age groups of African and Caliverde at the 14- and 21-day exposure periods.

The different mortality trends among these susceptible, tolerant, and resistant varieties account for the significant interaction of variety×plant age recorded in table 3. Though increased age undoubtedly provided increased vigor in all varieties, susceptible Caliverde and African failed to respond as well as the others. Growth in these varieties was accompanied by a corresponding increase in aphid population, thus offsetting greater tolerance associated with older, better established plants. Greater vigor in older plants of resistant varieties was clearly seen, probably because of greater manifestation of all three resistance bases—antibiosis, tolerance, and low preference for feeding. The importance of tolerance in resistant varieties is shown by their support of rather high aphid populations. After the death of the foliage of susceptible plants the root systems soon became inviable, producing no new crown growth, and rotting rapidly.

Discussion.—In interpreting these results it should be recognized that conditions of plant exposure to aphid attack were quite unlike those occurring in the field. Normal predator populations in uncaged plantings would probably retard the population increase. Cooler weather conditions might also alter the comparative patterns of plant mortality among the four varieties, as larger numbers of spotted alfalfa aphids can survive on resistant plants at lower temperatures (Howe & Smith 1957). It is therefore probable that seedling mortality would be greater among resistant varieties and that susceptible varieties would be killed more slowly. These data support field observations where susceptible varieties in both the mature and seedling stages have been destroyed by severe epidemics whereas mature stands of resistant Lahontan have been practically immune to kill. The rapid increase in resistance by Lahontan and Moapa during the early stages of growth, demonstrated here, should assist tremendously in the establishment of new stands, particularly under warm growing conditions similar to those occurring during this test.

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Soil Injection as a Means of Applying Systemic Acaricides to Fruit Trees[1]

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Abstract

Field trials in 1957 of the injection of 6 gallons of water containing 9 ounces of demeton, phorate, formerly designated Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate), or Chipman R-6199 (*O,O*-diethyl *S*-(2-diethylamino)-ethyl phosphorothioate [hydrogen oxalate salt]), into the soil under mature Grimes apple trees indicated that mites on apple trees might be controlled by this method of using systemic acaricides. Further studies at two locations in 1958 demonstrated that phorate was very effective when used in this manner and was superior to Chipman R-6199. Two devices for making such injections are described.

Injections with a hypodermic syringe of systemic acaricides into small pots containing lima bean plants gave excellent control of the two-spotted spider mite, *Tetranychus telarius* (L.), under laboratory conditions early in 1957. Studies were therefore undertaken to determine whether it would be possible to control the two-spotted spider mite and the European red mite, *Panonychus ulmi* (Koch), on fruit trees in a similar manner.

Materials and Methods.—A special soil-injection device (fig. 1) had to be designed before the acaricides could be adequately tested on fruit trees growing under natural conditions. The first one consisted of a 4-foot section of ⅜-inch galvanized pipe, treaded for a cap on one end and equipped with a solid point on the other. In the 6-inch area immediately above the point eight 3/32-inch orifices were drilled. The acaricide was forced into the pipe by air pressure from the supply tank through a hose connection provided near the threaded end.

The acaricides used in 1957 were demeton (28.5% EC),

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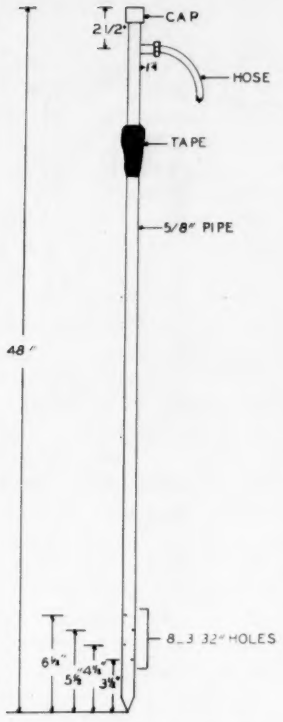

FIG. 1.—Air-pressure device for injecting systemic acaricides into soil.

phorate, formerly designated Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate) (47.5% EC), and Chipman R-6199 (*O,O*-diethyl *S*-(2-diethylamino)-ethyl phosphorothioate (hydrogen oxalate salt)) (72% water-soluble). Preliminary tests with 2 ounces of toxicant per 100 gallons of water applied to lima beans at 25 ml. and to potted roses at 100 ml. per plant caused no ill effects on the plants.

Before mites became active, the soil around small, non-bearing apple and pear trees planted close together on the laboratory grounds was treated by injection of 4 gallons of a mixture containing 8 ounces of toxicant per 100 gallons of water to a depth of 2½ feet at an air pressure of 45 p.s.i. No adverse effects from the acaricides were noted. However, the root systems of the trees had become interwoven, so that it was impossible to maintain a valid check tree. The mite population was sampled at intervals during the season and found to be extremely low. Attempts to increase it by placing two-spotted spider mites on the trees were unsuccessful. Indications were that the acaricides were at least partially responsible for the population suppression.

1957 Test.—The first orchard trial of the injection technique was begun on July 10, 1957 on Grimes apple trees at Vincennes, Indiana. Each insecticide was injected into the soil under one tree. Three trees were treated with different insecticides and one untreated tree served as a check. Three ounces of the toxicant in 2 gallons of water were injected 2 feet into the soil in each of three locations, at an air pressure of 47 p.s.i. The results summarized in table 1 indicate that mite control on apples might be accomplished with systemic insecticides injected into the soil under the trees.

1958 Tests.—This study was continued at the Purdue experimental orchard near Orleans, Indiana, in the spring of 1958, and in the O'Neil orchard near Vincennes in the summer of 1958. Trees in both orchards were set approximately 40 feet apart.

An improved injector (fig. 2) was constructed to operate from a conventional high-pressure hydraulic sprayer and attached to the hose in the same manner as a spray gun. It consisted of a 4-foot section of ¼-inch pipe inside ¾-inch pipe of the same length. The two pipes were welded together and closed at one end. This end was fashioned into a point and a 1/16-inch opening drilled from the point to the inner pipe. The outer pipe was provided with eight 3/32-inch orifices near the point, and both pipes had individual cutoff valves. A Y led from these valves to the hose attachment.

The injector was inserted into the soil. At about 100 p.s.i. with the outer pipe closed, a strong jet of fluid was produced through the inner pipe at the tip which acted as a water drill, allowing the apparatus to be placed into the soil with little effort. After the device had been inserted about 3 feet, the inner pipe was closed and the outer one opened, allowing the fluid to move out horizontally through the soil. It was found that the pressure used to effect this horizontal movement had to be varied with soil conditions. It was usually about 50 p.s.i.

Treatments in the Purdue orchard were on Red Delicious, Winesap, and Jonathan varieties and were as follows: (1) check, water alone injected into soil; (2) phorate (90% EC) 12 ounces per 100 gallons; (3) Chipman R-6199 (71% water-soluble) 15.3 ounces per 100 gallons. Injections were at the rate of 36 gallons per tree, 9 gallons at each of four locations under a tree. Treatments were applied on April 23.

Treatments in the O'Neil orchard were on Grimes variety and were as follows: (1) check, no treatment; (2) phorate (90% EC) 12 ounces per 100 gallons; (3) Chipman R-6199 (71% water-soluble) 16 ounces per 100

Table 1.—Effectiveness of soil injection of systemic acaricides against the two-spotted spider mite and the European red mite on apples, O'Neil orchard, Vincennes, Ind., 1957.

	Number of Mobile Mites per 100 Leaves													
	July 10		July 17		July 24		July 31		August 14		August 27		September 11	
Treatment (July 10)	Spider Mite	Red Mite	Spider Mite	Red Mite	Spider Mite	Red Mite	Spider Mite	Red Mite	Spider Mite	Red Mite	Spider Mite	Red Mite	Spider Mite	Red Mite
Untreated	90	360	500	890	485	210	240	205	510	280	195	145	70	184
Demeton	30	410	45	410	8	20	7	41	16	28	90	170	42	350
Phorate	80	540	30	1,590	30	160	1	35	5	22	5	165	58	226
Chipman R-6199	10	580	70	1,810	5	46	20	35	20	42	15	130	14	168

Table 2.—Effectiveness of soil injection of systemic acaricides against the European red mite on apples, Purdue orchard, Orleans, Ind. 1958.

Treatment (April 23) and Variety	Number of Mobile Mites per 100 Leaves				
	April 30	May 14	May 28	June 11	June 25
Checks (water only):					
Red Delicious	210	140	1,420	7,640	17,640
Winesap	180	60	800	1,310	3,780
Jonathan	60	20	150	500	1,500
Average	150	70	790	3,150	7,640
Phorate:					
Red Delicious	240	80	330	1,160	1,480
Winesap	180	20	170	190	1,110
Jonathan	30	30	100	540	1,860
Average	150	40	200	630	1,480
Chipman R-6199:					
Red Delicious	160	70	410	3,260	11,280
Winesap	30	70	640	1,320	3,660
Jonathan	40	10	90	710	2,310
Average	80	50	380	1,760	5,750

Table 3.—Effectiveness of soil injection of systemic acaricides against the European red mite on apples, O'Neil orchard, Vincennes, Ind. 1958.

Treatment (June 12)	Number of Mobile Mites per 100 Leaves						
	June 12	June 19	June 26	July 10	July 24	August 7	August 21
Check (untreated)	195	285	360	250	5,660	3,900	170
Phorate	156	10	6	0	39	22	0
Chipman R-6199	110	230	115	705	11,720	2,320	30

gallons. Injections were at the rate of 120 gallons per tree, 10 gallons at each of 12 locations under a tree. Treatments were applied on June 12.

RESULTS.—The results of the experiments are summarized in tables 2 and 3. The injection application of phorate gave a very effective reduction in the mite population in both the Purdue and O'Neil orchards. Similar application of Chipman R-6199 was less promising, effecting only a small reduction in the Purdue orchard and practically none in the O'Neil orchard.

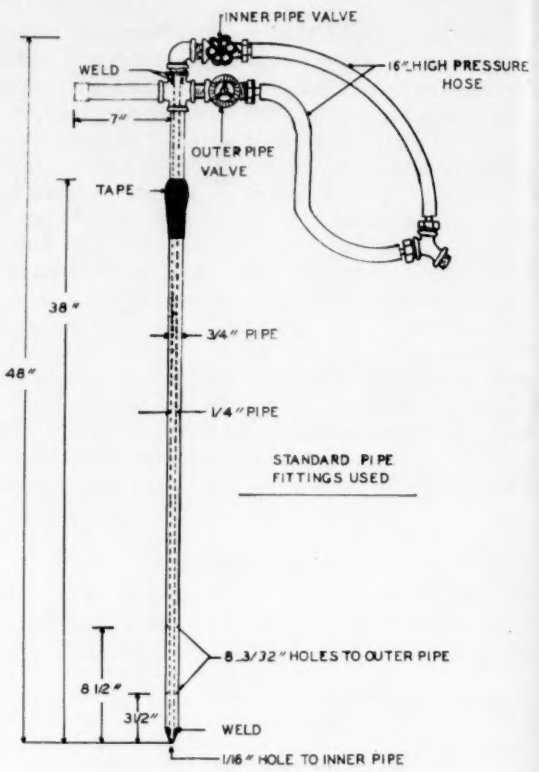

FIG. 2.—Water-pressure device for injecting systemic acaricides into soil.

A Biotype of the Spotted Alfalfa Aphid on Alfalfa[1]

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ABSTRACT

In February 1958, near El Centro, California, symptoms of susceptibility to the spotted alfalfa aphid (*Therioaphis maculata* (Buckton)) were observed in the previously resistant alfalfa clone N(evada)-1018. A series of tests from March to October demonstrated that a biotype, designated ENT A, was capable of surviving and reproducing on three of the eight parent clones of the aphid-resistant nondormant variety Moapa. The new biotype did not show a similar reaction to five other Moapa parent clones tested or to any of the five parent clones of Lahontan, a variety also resistant to this aphid. Comparisons were made with a standard working culture of the aphid, designated ENT B, originated and maintained at Bakersfield, California. In a cage test of Moapa as a variety grown from breeder seed, biotype ENT A produced much higher populations but plant mortality was not increased. Little damage to Moapa should occur unless or until biotype ENT A becomes dominant in the aphid population of an area. Even so, populations on Moapa can be expected to be much lower than those that develop on susceptible varieties

In February 1958, in the alfalfa nursery at the Imperial Valley field station, University of California, near El Centro, the alfalfa clone N(evada)-1018 showed symptoms of susceptibility to the spotted alfalfa aphid (*Therioaphis maculata* (Buckton)). In the preceding 3 years this clone had been present during repeated and prolonged severe nursery infestations of this pest without showing any such symptoms. Furthermore, N-1018 was included in a number of stem-cage tests by personnel of the Entomology Research Division, in which it was rated as resistant or highly resistant to this aphid, and had subsequently been selected as one of the nine parent clones of the resistant variety Moapa. The occurrence of susceptibility symptoms at this time therefore indicated development of a biotype capable of reproducing on N-1018 and

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perhaps on other clones rated as resistant to the spotted alfalfa aphid.

Although the spotted alfalfa aphid has been known in this country only since 1954, when an economic infestation was discovered in New Mexico, indications of the development of biotypes have already been noted. Stern & Reynolds (1958) reported on its resistance to organophosphorus insecticides in southern California and suggested that this resistance had resulted from mutation after the aphid arrived in North America. Dickson *et al.* (1958) concurred with the theory that the strain developed by mutation and subsequent selection in southern California. They suggested that this aphid, being entirely parthenogenetic, should be slow to change and that the development of superresistant populations should be delayed unless they came about through the mutation of a new single gene for superresistance.

In 1957 Stanford & McMurtry (1959) found indications of a biotype of the spotted alfalfa aphid that could reproduce at a more rapid rate than aphids from two other cultures on the two parent clones of the variety Lahontan that are rated intermediate in resistance to this pest. They observed, however, that the danger of development of a biotype able to reproduce on a highly resistant plant is diminished by the fact that the reproductive rate is lower on intermediate than on susceptible plants. They also suggested that predators might contribute to prevention of a virulent biotype by being better able to keep up with the aphid populations in plantings of resistant varieties.

In November 1957 a standard working culture of the spotted alfalfa aphid was established at the Bakersfield station of the Entomology Research Division from aphids then infesting the station nursery. A series of tests to compare the aphids discovered thriving on N-1018 with the normally responding aphids of the Bakersfield culture was carried out during the 1958 season. This paper reports the results of these tests and gives a preliminary evaluation of the importance of this development in relation to currently available resistant varieties.

ANTIBIOSIS TESTS.—*At El Centro Nursery.*—An initial stem-cage test was undertaken at the El Centro nursery in March 1958. Eight alfalfa clones were selected for inclusion in this test: namely, two Lahontan clones, C-84 and C-89; a Moapa clone, N-1018; three progeny of C-84—Meloland 2-5, Meloland IP-6-14, and Meloland IP-35-6; one progeny of N-1018, Meloland IP-124-4, and a spotted alfalfa aphid susceptible selection from the variety African, Meloland AW-35. Aphids were removed from an infested N-1018 plant in the nursery and transferred to a caged potted susceptible plant in the greenhouse to establish a new culture, designated ENT A. The Bakersfield culture was then designated ENT B, and a supply was identically maintained for use in this test. In addition, random collections were made from a bed of susceptible African plants located in the windward corner of the nursery and in a commercial stand of African near Holtville, these two collections being similarly established on potted plants and designated respectively as cultures ENT C and ENT D. These four cultures were compared simultaneously on each of the selected clones. The cages and methods used were essentially those described by Howe & Smith (1957).

Results of this test are given in table 1. Aphids of culture ENT A became readily established on N-1018 and Meloland IP-124-4, which has N-1018 parentage, but not on C-84 or C-89 or on the plants having C-84 in their parentage. Aphids of the other three cultures responded normally to all the resistant plants.

At Bakersfield Nursery.—To verify the results of the test at El Centro and to determine whether culture ENT A was able to establish itself on other parent clones of Moapa, two tests were undertaken at the Bakersfield nursery, one in May and one from mid-June to mid-July. Eight of the nine parent clones of Moapa were present in the Bakersfield nursery; all were included. These eight Moapa clones were N-1018, N-1032, N-1041, N-1047, N-1059, N-1066, N-1074, and N-1075. The four aphid cultures used at El Centro were transported to Bakersfield and increased on highly tolerant, but susceptible plants in the clonal beds.

The first test was conducted in the same manner as the one at El Centro, except that five nymphs were introduced initially, as compared with three at El Centro. The data obtained (table 1) present a sharp contrast between aphids from culture ENT A and those from the other three cultures in ability to become established on three of the Moapa clones. Aphids from ENT A readily became established on clones N-1018, N-1066, and N-1075, but reacted normally to the other five. Aphids from the other three cultures reacted normally on all eight Moapa clones. There appeared to be no important differences in reaction of aphids from any of the four cultures to the two Lahontan clones included in this test.

In the second test the procedure was altered. Introduced nymphs that survived were permitted to attain the fourth instar, at which time one nymph was retained to acquire her individual progeny record as an adult, and the others were discarded. Some of the retained aphids died in a short time, so that there was an opportunity to start one or two others. In this test all cages were examined daily to obtain maximum accuracy. Nymphs produced by each aphid retained for progeny record were also considered survivors and removed when they attained the fourth instar. Since cultures ENT C and D had exhibited no differences in comparison with ENT B in the previous tests, they were discarded, and only the permanent culture, ENT B, was used for comparison with ENT A. The data obtained in this test (table 2) substantiate those in the two earlier ones.

At this stage in the study it seemed advisable to test the five parent clones of Lahontan. This variety was developed for resistance to stem nematode but was observed to be resistant also to the spotted alfalfa aphid. Howe & Smith (1957) determined that the aphid resistance of the parent clones varied from high to intermediate. Accordingly, two tests were run, one in August and one in September. Results are summarized in tables 3 and 4. No differences in reaction between aphids from cultures ENT A and ENT B to any of the Lahontan clones, irrespective of resistance level, were demonstrated.

LARGE CAGE TEST OF MOAPA BREEDER SEED.—To obtain data on the effect of biotype ENT A on Moapa as a variety, a test was improvised using plants from breeder seed sown in rows for another purpose. Lumite-covered cages, 6×10 feet, were placed over rows of alfalfa, the

Table 1.—Survival and development of spotted alfalfa aphids collected from different cultures on resistant alfalfa clones. 1958.

Alfalfa Clone	Aphid Culture (ENT)	No. of Nymphs Introduced	No. of Nymphs Becoming Adult	No. of Progeny Observed: Nymphs	No. of Progeny Observed: Adults	No. of Progeny Surviving Two Instars
El Centro, California, March 12 to April 5						
C-84	A	12	0	0	0	0
	B	9	0	0	0	0
	C	12	1	1	0	0
	D	12	0	0	0	0
C-89	A	12	1	2	0	0
	B	3	3	7	0	0
	C	9	1	8	0	5[a]
	D	12	0	0	0	0
N-1018	A	9	6	32	0	31[b]
	B	6	1	2	0	0
	C	8	1	3	0	0
	D	8	2	1	0	0
Meloland AW-35	A	3	2	58	14	72[b]
	B	3	2	120	19	139[b]
	C	5	4	30+	11	41[b]
	D	3	1	37	5	42[b]
Meloland 2-5	A	10	0	0	0	0
	B	9	0	0	0	0
	C	12	0	0	0	0
	D	12	0	0	0	0
Meloland IP-6-14[c]	A	12	1	1	0	0
	B	3	1	10	0	2
	C	7	3	9	0	0
	D	10	1	2	0	0
Meloland IP-35-6[c]	A	12	0	0	0	0
	B	9	0	0	0	0
	C	10	1	0	0	0
	D	12	0	0	0	0
Meloland IP-124-4	A	9	1	33	12	45[b]
	B	6	4	7	0	3[a]
	C	11	1	0	0	0
	D	10	1	0	0	0
Bakersfield, California, May 6–13						
C-84	A	28	2	0	0	0
	B	22	3	0	0	0
	C	18	3	1	0	0
	D	19	1	0	0	0
C-89	A	18	8	18	0	0
	B	19	3	7	0	0
	C	17	5	5	0	3[d]
	D	22	2	0	0	0
N-1018	A	7	5	329	31	360[b]
	B	20	3	1	0	0
	C	24	1	0	0	0
	D	20	2	0	0	0
N-1032	A	21	5	16	0	0
	B	19	1	0	0	0
	C	18	4	1	0	0
	D	17	4	0	0	0
N-1041	A	20	5	2	0	0
	B	20	5	0	0	0
	C	20	3	2	0	0
	D	17	4	6	0	4[a]
N-1047	A	19	5	4	0	0
	B	15	5	5	0	0
	C	16	5	3	0	0
	D	15	4	3	0	0
N-1059	A	21	4	1	0	0
	B	19	4	9	0	0
	C	22	1	0	0	0
	D	16	4	11	0	8[a]
N-1066	A	5	5	395	40	435[b]
	B	20	3	16	0	16[a]
	C	26	0	0	0	0
	D	22	1	0	0	0

[a] All in second instar at conclusion of tests. It is doubtful whether these nymphs would have survived beyond this instar had the tests been continued.
[b] All instars at conclusion of test.
[c] Highly resistant backcross.
[d] African selection, intermediate in resistance.

Table 1.—(Continued)

Alfalfa Clone	Aphid Culture (ENT)	No. of Nymphs Introduced	No. of Nymphs Becoming Adult	No. of Progeny Observed: Nymphs	No. of Progeny Observed: Adults	No. of Progeny Surviving Two Instars
	Bakersfield, California, May 6–13					
N-1074	A	24	6	10	0	1[a]
	B	19	3	2	0	0
	C	20	2	2	0	2[a]
	D	17	1	5	0	5[a]
N-1075	A	6	5	220	31	251[b]
	B	19	4	5	0	2[a]
	C	24	1	0	0	0
	D	18	1	0	0	0
N-1188[d]	A	13	8	98	11	94[b]
	B	9	7	23	0	3
	C	14	9	33	0	13[a]
	D	11	5	35	0	9

growth was cut back, and the caged areas were sprayed twice with TEPP, 2 days apart, to kill any aphids present. As soon as a few inches of regrowth appeared, the areas covered by the cages were examined, to be certain they were free or approximately free of aphids. Then each area was infested with aphids from one culture or the other. Populations increased readily on the two or three rows of susceptible material in all cages, and by August 27, when the populations on resistant varieties were measured, most of the susceptible material was dead or dying. The test was severe, and the results should be considered in this light until data from open field plantings are available.

Table 5 summarizes the data considered pertinent to this paper. It will be seen that biotype ENT A produced a higher population on Moapa (75% of stems with an economic number of aphids), but that this higher infestation, accompanied, of course, by more honeydew, did not increase mortality among the 6-month-old plants. However, the effect of biotype ENT A on Zia, an aphid-resistant variety released by New Mexico, appeared to be decidedly more severe than that of biotype ENT B.

DISCUSSION.—The data provided by these tests were obtained under various weather conditions (table 6) and at two distinctly different locations. Although weather in the Southwest tended to be cooler and wetter than normal early in 1958, conditions experienced at Bakersfield, particularly in the third and fourth tests, were ideal for spotted alfalfa aphid development. It is well known that weather may play an important role in plant resistance to an insect, and that a number of environmental conditions can produce temporary abnormal insect reactions. The consistency of the test results testifies to the validity of the contention that a biotype of the spotted alfalfa aphid capable of surviving on previously resistant, nondormant alfalfa clones has appeared. Furthermore, there seems to be no evidence that disease or physiology of the host plant accounts for the survival and reproduction of certain aphids on the several clones involved. Outdoor stem cage tests have proved very satisfactory for obtaining reliable resistance data. Stock cultures of biotypes ENT A and ENT B were maintained on clones of a highly tolerant but otherwise susceptible alfalfa selection in the Bakerfield clonal nursery. They were therefore exposed to similar environmental conditions. To minimize the effects of a so-called temporary conditioning resulting from previous foods or environmental conditions, aphids

Table 2.—Survival and reproductive capacity of spotted alfalfa aphids collected from different sources on resistant alfalfa clones, Bakersfield, California, June 13 to July 14, 1958.

Alfalfa Clone	Aphid Culture (ENT)	Number of Nymphs			Progeny Dying Before		
		Introduced	Attaining Fourth Instar	Kept for Progeny Record	Average No. of Progeny per Adult[a]	Fourth Instar (%)	Average Reproduction Period
C-84	A	40	0	0	—	—	—
	B	35	4	2	0	—	—
C-89	A	11	6	3	5	100	2
	B	11	7	2	3.5	86	2
N-1018	A	4	4	1	53	0[b]	21
	B	47	0	0	—	—	—
N-1032	A	9	5	2	2.5	100	2.5
	B	18	3	1	4	100	4
N-1041	A	8	3	1	15	67	11
	B	21	5	1	0	—	—
N-1047	A	13	3	2	6.5	100	7.5
	B	30	3	0	—	—	—
N-1059	A	28	3	0	—	—	—
	B	23	4	1	7	100	4
N-1066	A	3	3	1	110	0	29
	B	38	1	0	—	—	—
N-1074	A	28	3	0	—	—	—
	B	27	0	0	—	—	—
N-1075	A	4	2	1	15	13	9
	B	38	1	1	3	100	1
N-1188[c]	A	5	3	1	46	22	13
	B	4	4	1	77	42[b]	21
No. Car. 1014-4C	A	43	1	0	—	—	—
	B	43	0	0	—	—	—
Caliverde	A	3	3	1	83	0	18
	B	4	3	1	102	0	19

[a] Based on nymphs observed during daily examination of cages.
[b] Predator in cage destroyed adult and/or nymphs; tests then discontinued.
[c] African selection, intermediate in resistance.

Table 3.—Comparison of survival and reproduction of spotted alfalfa aphids from cultures of biotypes ENT A and ENT B on the five parent clones of Lahontan, Bakersfield, California, August 1958.

Alfalfa Clone	Aphid Culture (ENT)	Number of Nymphs		Number of Progeny Observed[a]		
		Introduced	Becoming Adult	Total	Surviving Second Instar	Becoming Adult
C-84	A	25	0	—	—	—
	B	25	0	—	—	—
C-89	A	14	2	15	4	2
	B	14	2	4	1	0
C-900	A	20	1	2	0	—
	B	20	1	0	—	—
C-901	A	4	2	286		
	B	5	3	351		
C-902	A	4	3	246		
	B	7	4	26[b]		
Caliverde	A	3	3	3,596		
	B	3	3	2,961		

[a] All reproduction occurred between August 1 and 19.
[b] Lost in windstorm accident on August 15; reproduction comparable to that of ENT A aphids at the time.

used in tests were from successive generations of these stock cultures. Identity of biotype ENT A stock cultures was confirmed by returns of randomly selected individuals to N-1018.

Painter (1951) states that there appear to be two general types of biological races (biotypes) in insects so far as resistance in plants is concerned. One type, represented by the pea aphid, *Macrosiphum pisi* (Harr.), on peas (Harrington 1945), is a larger more vigorous strain. In the second type Painter suggests that certain elements of the insect physiology must fit specific elements of the plant physiology as a key fits a lock; *i.e.*, an adjustment by the insect to conditions resulting from the presence of a particular gene in the plant. Simultaneous observations of the fecundity rates of biotypes ENT A and ENT B were made beginning in mid-October up to January 9, 1959. The average number of nymphs per reproductive day produced by 10 adults of biotype ENT A was 1.78, and by 10 adults of biotype ENT B, 1.71. The lack of difference in fecundity rates supports the conclusion that ENT A is not a more vigorous strain than ENT B. The complete

Table 4.—Comparison of survival and reproduction of spotted alfalfa aphids from cultures of biotypes ENT A and ENT B on the five parent clones of Lahontan, Bakersfield, California, September 1958.

Alfalfa Clone	Aphid Culture (ENT)	Number of Nymphs		Progeny Observed[a]	
		Introduced	Becoming Adult	Total	Present at Close of Test
C-84	A	31	0	—	—
	B	35	0	—	—
C-89	A	25	0	0	—
	B	17	1	2	0
C-900	A	8	2	10	3
	B	18	1	4	4
C-901	A	4	3	19	19
	B	6	4	48	48
C-902	A	3	3	21	21
	B	7	3	18	18
N-1018	A	8	2	21	21
	B	32	0	—	—
N-1018	A	9	4	30	30
	B	33	0	—	—
Caliverde	A	3	3	113	—[b]
	B	2	2	95	—[b]

[a] All reproduction occurred between September 17 and 26.
[b] Older nymphs removed periodically to prevent occurrence of adult progeny on plant.

Table 5.—Aphid infestation and plant mortality in rows of alfalfa exposed to two biotypes of the spotted alfalfa aphids, Bakersfield, California, August 1958.

Alfalfa Variety	Biotpye (ENT)	No. of Aphids per Stem		Stems Having 30+ Aphids (%)	Number of Plants Exposed to Aphids	
		Range	Average		Total	Killed
Moapa	A	5–366	96.5	75	29	0
		5–705	196.2	75	30	3
	B	0–109	22.3	29	39	0
		0–153	27.9	21	34	1
Caliverde	A	—	—	—	28	27
	B	—	—	—	35	33
Zia	A[a]	2–192	38.4	46	30	20
	B	0–112	14.2	17	31	9

[a] Only scanty, short growth; remaining population probably much less than maximum developed.

lack of antibiosis to ENT A in the alfalfa clones N-1018, N-1066, and N-1075 indicates that this biotype represents the second described by Painter.

In an unpublished report on the evaluation of resistance to the spotted alfalfa aphid in Moapa, Howe and Pesho mentioned that N-1018, N-1041, N-1066, and N-1075 when tested in cool weather in 1956 at Anaheim, California, permitted some aphids to mature and to reproduce at very low rates. Although no relationship has been established between this experience and the development of ENT A, it may be more than coincidence that three of these four clones now display susceptibility to this biotype.

Table 6.—Temperature and precipitation records for periods when stem-cage tests were conducted, El Centro and Bakersfield, California, 1958.[a]

Period	Temperature (° F.)	Days in Test Period				Rainfall (Inches)
		Total	When Temperature Exceeded			
			80° F.	90° F.	100° F.	
	El Centro					
March 12–April 5	39– 82	25	1	0	0	0.6
	Bakersfield					
May 6–23	48– 98	18	14	10	0	0.7
June 6–July 14	55–107	39	39	29	11	0
July 31–August 15	63–106	16	16	16	11	0
September 11–26	50–100	16	14	8	0	0.4

[a] Data from Weather Bureau, El Centro airport, and Kern County Land Co., at Bakersfield.

At present the importance of biotype ENT A relates to the new variety Moapa. Just how much Moapa will be affected will not be determined until this biotype is observed in open commercial plantings. However, it appears safe to assume that little or no damage will be encountered unless or until ENT A becomes dominant in the population of an area. Furthermore, it is doubtful that infestations as large as that produced in the cage test reported in table 5 will develop in open plantings. Even so, this population would be far below that which develops on susceptible varieties. At present the new strain appears to be scarce or absent in the area surrounding its probable place of origin. A limited survey consisting of samples of aphids from 18 fields in the vicinity of Yuma, Arizona, and throughout Imperial Valley, California, taken October 1, 1958, failed to produce individuals indentifiable as biotype ENT A by confinement on clone N-1018. The three clones susceptible to this biotype are present in several nurseries in California, Nevada, and Arizona, but no other infestations or damage to these clones have been reported.

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Diapause and Secondary Parasites Nullify the Effectiveness of Rose-Aphid Parasites[1] in Riverside, California, 1957–1958[2,3]

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ABSTRACT

The disadvantageous effects of facultative diapause in the following five species of aphidiine parasites of the rose aphid, *Macrosiphum rosae* (L.), are discussed: *Aphidius nigripes* Ashmead, *A. alius* Muesebeck, *A. confusus* Ashmead, *Praon occidentalis* Baker, and *P. unicus* Smith. Because of the availability of aphidiine cocoons (diapause and nondiapause) throughout the season, secondary parasitism by *Asaphes californicus* Girault, *Pachyneuron siphonophorae* (Ashmead), *Aphidencyrtus aphidivorus* (Mayr), and several *Charips* species is shown to be detrimental to the effectiveness of the primary parasites. The effect of other natural enemies of the rose aphid in competition with the aphidiines is also noted.

It is well known that diapause may enable an organism to overcome adverse conditions. Schlinger & Hall (1959 and 1960a) recently discussed facultative diapause in *Praon palitans* Muesebeck, and van den Bosch *et al.* (1959) reported that diapause in this parasite is an important factor affecting biological control of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), in the colder areas of southern California. In the case of *P. palitans* the diapause of the parasite is perfectly synchronized with the aphid biology, although the aphid apparently did not enter diapause. The types of dispause (partial and complete), their frequency, and general distributional patterns in *P. palitans* have been reported in more detail by Schlinger & Hall (1960a). A more thorough discourse on diapause in the Aphidiinae is now in preparation by the author.

Observations and rearings of various aphid parasites have revealed that many of the Aphidiinae have the ability to undergo a diapause condition either in winter or in summer, or, in a few cases such as *Praon simulans* (Provancher), both in summer and in winter (Schlinger & Hall 1960b).

This discussion is an attempt to answer the following question: Why is the rose aphid not under effective natural control in Riverside, California? It presents the results of observations made on the rose aphid, *Macrosiphum rosae* (L.), and its parasites in Riverside, California, during 1957 and 1958.

METHODS.—Parasitized aphids were collected from unsprayed rose plants grown in various home gardens. The unvacated cocoons as they were found attached to the stems or leaves were taken to the laboratory and placed in cylindrical half-pint ice-cream cartons capped with petri dishes. The laboratory was maintained at a temperature of 70° to 75° F., and at 50% to 60% R. H. These conditions were considered satisfactory for continuous rearing, since many other species of aphid parasites, including *Praon palitans*, were thus being propagated without showing any sign of diapause.

Both primary and secondary parasites were removed from the containers immediately upon emergence. This procedure is particularly important in the case of the secondary parasites, to preclude any reparasitization of the primary diapause larvae or pupae. Dissections of unvacated aphidiine cocoons were made at intervals of 1, 3, and 6 months after a preliminary peroid of at least 2 weeks to allow for the emergence of nondiapause individuals, which always emerged within 10 days after placement in the laboratory.

Most of the field observations were made between March 15 and May 15, since it was during this period that the rose aphid was present in Riverside, California, in sufficient numbers for this study.

THE PARASITES.—The five species of primary parasites reared from the rose aphid in Riverside were *Aphidius nigripes* Ashmead, *A. confusus* Ashmead, *A. alius* Muesebeck, *Praon occidentalis* Baker, and *P. unicus* Smith. All these species except *A. confusus* apparently utilize the rose aphid to some degree as one of their hosts. The observations discussed below deal with only four of these species, since *A. confusus* was not reared in large enough numbers to determine its real diapause condition. Since all four species are similar in their diapause responses, this discussion relates to all the species, hereafter cited as *Aphidius* spp. and *Praon* spp.

The secondary parasites reared from the above-named primary parasites were *Asaphes californicus* Girault, *Pachyneuron siphonophorae* (Ashmead), *Aphidencyrtus aphidivorus* (Mayr), and several undetermined *Charips* species.

HOSTS OF PRIMARY PARASITES.—Each of the four primary parasites has been reared from one or more hosts other than the rose aphid. Some, if not all, appear to prefer other hosts, as with *Praon occidentalis* and *P. unicus*, which attack the pea aphid (*Macrosiphum pisi* (Harris)) and the ambrosia aphid, respectively.

The author observed that none of these parasites is specific to or even prefers the rose aphid as a host, and, unlike the host in southern California, none is subject to diapause. It is therefore easy to understand the inability to attain a large degree of natural control of this host. The fact that the rose aphid is apparently not a preferred host, together with the parasite's subsequent diapause and the consequent heavy attack by secondary parasites, explains this ineffective control, as discussed below.

HISTORY OF A TYPICAL PARASITE.—Between February 1 and March 1, winged rose aphids begin to appear on the newly formed rose shoots. As temperatures begin to rise in late February and early March many young aphids are produced. The parasites, during the early part of this period, are for the most part diapause larvae in their

[1] Hymenoptera: Braconidae, Aphidiinae.

[2] Paper No. 1178, University of California Citrus Experiment Station, Riverside, California. Accepted for publication September 8, 1959.

[3] This report does not include two species of the eulophid genus *Aphelinus* which also exhibited diapause.

[4] The author is grateful to Dr. C. F. W. Muesebeck and Dr. B. D. Burks, both of th U. S. Department of Agriculture, Washington, D. C., for identification of primary and secondary parasites, respectively; and to Mr. J. C. Hall of the University of California Citrus Experiment Station, Riverside, for technical assistance in the laboratory.

cocoons. They begin to emerge as adults about February 15. Cocoons formed by their F_1 progeny are usually noticeable by March 15. As temperature and daylight continue to increase during the spring, the rose aphid becomes more and more abundant, so that by the end of March its populations have become large enough to permit considerable predator activity by syrphid, coccinellid, and *Chrysopa* larvae.

At this same time the rose stems grow more rapidly. The aphids tend to remain attached to the new leaflets as they expand, rather than to move away and up the new succulent stems. Thus the aphids that were parasitized earlier on the succulent terminal buds are subsequently found several inches down the stem, continuing their feeding on the now older leaves and stems. By carefully collecting cocoons from the lowermost leaves and stems only, and comparing the percentage emergence with that of the same species collected from the uppermost leaves, it was invariably found that those cocoons from the lower leaves contained only diapause individuals, while those from the upper leaves contained nondiapause as well as diapause individuals. This condition occurred in the first generation of parasites. With each succeeding generation the percentage of individuals entering diapause increased, as shown in table 1.

The F_2 generation of parasites usually has a preponderance of males, the rearings often showing more than 70%. This percentage, however, allows for good mating for the next generation and for its progeny, so that actually a good proportion of the next complete diapause generation is composed of females.

Around April 1, the second generation of parasites is under way, but because at least half the first generation already has entered diapause, its reproductivity is about half that which would otherwise occur (table 1). Although the predator populations have increased, most roses have been sprayed at least twice. This treatment, together with diapause, certainly has a detrimental effect on the action of any living, nondiapausing parasite. In unsprayed rose gardens, however, the aphid has now reached great abundance, and any large degree of natural control that occurs at this time is apparently an effect of the predators. Another factor which adversely affects the parasite at this time, is the cutting of roses for use elsewhere, thus the parasitized aphids and the parasite cocoons are removed from the host's environment.

By the end of April the third generation of parasites is present, but instead of increasing in great numbers and effecting considerable control of the aphid, only about 25% of the reproductivity that would be expected in the absence of the adverse factors noted above is available (table 1; fig. 1). Usually, beginning early in June, most of the aphid populations on the roses decline, presumably owing to a combination of abiotic and biotic factors, including the physiological condition of the plant and of the aphid. The fourth generation of parasites is a poor one in regard to any natural control of the aphids, and the number of parasites entering diapause has increased to nearly 100%. By this time parasite reproductivity is only about 12% of the estimated potential parasitism. Apparently, all individuals from the fourth and fifth generations (where a fifth occurs) enter diapause (table 1; fig. 1).

Usually by late fall or winter all the diapause cocoons have fallen to the ground with the leaves. The parasite is then subjected to still another adverse factor, since nearly all rose gardens examined showed that the dead leaves containing the diapausing parasites were removed by "clean culture" from the vicinity of the rose plants and hence of their host, the rose aphid. The parasite larvae normally remain in diapause in the cocoons through the summer and winter and emerge after about 8 months in diapause. However, a few individuals have been observed to emerge during warm periods of the winter only 6 months after entering diapause.

EFFECT OF COMPETITIVE NATURAL ENEMIES.—Two groups of secondary parasites attack the rose-aphid parasites. Parasites of the first group, which includes the pteromalids *Asaphes californicus* and *Pachyneuron siphonophorae*, attack the primary parasites *Aphidius* spp. and *Praon* spp. and emerge readily from the nondiapause and

Table 1.—Diapause and emergence of field-collected primary and secondary parasites of *Macrosiphum rosae*.

Generation of Parasite	Date of Sampling (1958)	No. of Cocoons	Emergence of Parasites						Individuals Entering Diapause[a]	
			Primary		Secondary		Total			
			No.	%	No.	%	No.	%	No.	%[b]
1	3/15	12	5	41	1	8	6	49	6	51
	3/21	23	2	8	9	39	11	47	12	53
2	3/24	22	3	13	3	13	6	26	16	74
	4/4	25	6	24	4	16	10	40	15	60
	4/10	14	2	14	9	64	11	78	3	22*
3	4/17	41	3	7	15	36	18	43	23	57
	4/24	60	6	10	4	6	10	16	50	84
	4/30	10	0	0	7	70	7	70	3	30*
4	5/15	8	0	0	1	12	1	12	7	88
	5/30	1	0	0	0	0	0	0	1	100
Totals (Nos.) or Averages (%)	—	216	27	*12*	53	*26*	80	*38*	136	*62*

[a] Diapause individuals were calculated by subsequent dissection of unvacated cocoons.
[b] Diapause percentage was always larger than shown, since certain secondary parasites were able to develop and emerge from diapause cocoons. This was particularly true of those samples marked with an asterisk (*).

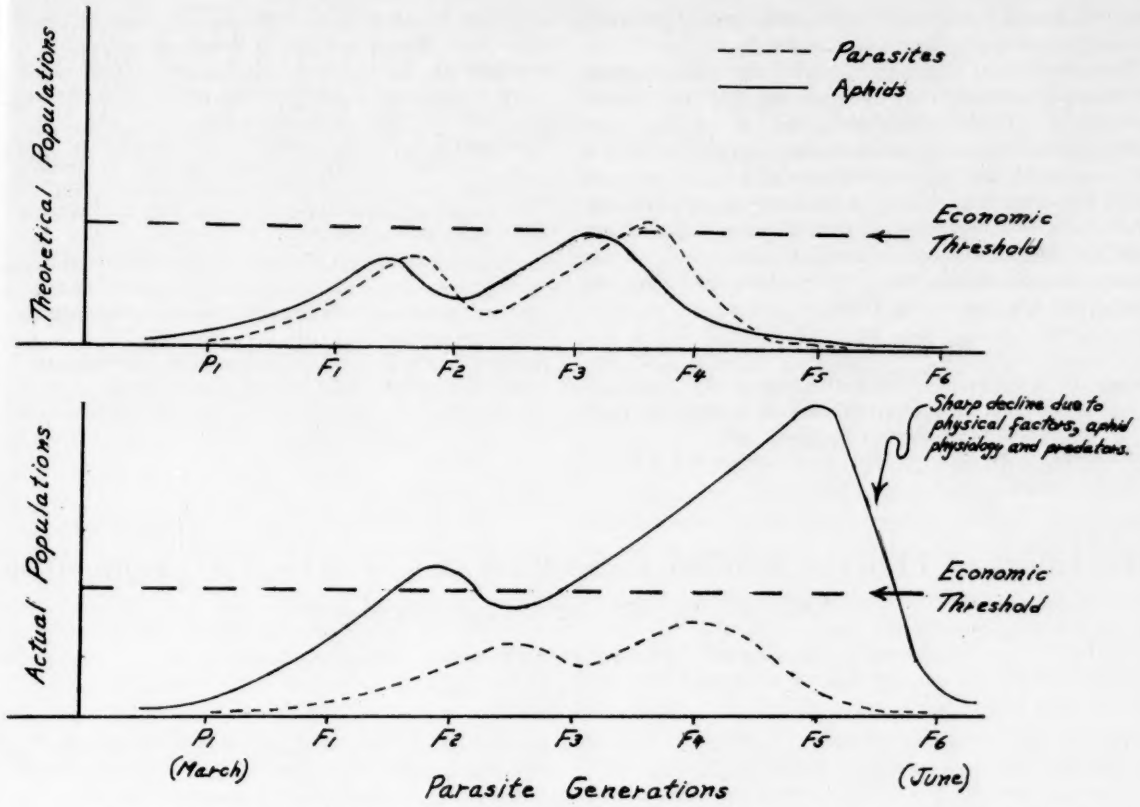

FIG. 1.—Actual and theoretical population curves of the rose aphid, *Macrosiphum rosae* (L.), and its parasites.

diapause cocoons of both hosts. The second group includes several species of the cynipid genus *Charips* and the encyrtid *Aphidencyrtus aphidivorus*, both of whose larvae attack their hosts when the latter are in either their nondiapause or their diapause cocoons. Emergence of these secondaries during the season is from the nondiapause cocoons only.

Since large numbers of diapause cocoons, as well as smaller numbers of nondiapause cocoons, are available throughout the season in which these secondary parasites may oviposit, the result is abundant hyperparasitism of the aphidiine populations. This condition is similar to the case cited by Clancy (1944) for the primary parasite *Clausenia purpurea* Ishii, which was attacked by a secondary *Lygocerus* sp. early in the year before *C. purpurea* was able to emerge. In this way the *Lygocerus* sp. obtained a "free" generation on its host, and Clancy noted this as an important factor inhibiting the effectiveness of *C. purpurea* on its host, *Pseudococcus comstocki* (Kuw.), in Virginia.

Since none of these secondary parasites is known to be specific to any aphidiine species as noted by Schlinger & Hall (1960b) and as indicated also by the numerous host records cited by Muesebeck *et al.* (1951), the rose-aphid parasites are often heavily attacked by secondary parasites. These build up in great numbers on the nearly omnipresent *Aphidius* (*Lysiphlebus*) *testaceipes* (Cresson), and therefore are able to attack the rose-aphid parasites throughout the entire rose-aphid season. Also, the emergence of *Charips* and *Aphidencyrtus* in the spring at about the same time as the emergence of the diapause *Aphidius* spp. and *Praon* spp. permits a concerted and immediate attack on the F_1 aphidiine generation.

It may be concluded that because of the high proportion of diapause individuals in the populations of rose-aphid parasites, the secondary parasites have a marked detrimental effect on the primary parasites. This fact is pointed out clearly in table 1. In fact, the heavy attack by the secondary parasites has made it exceedingly difficult to rear any large series of these primary parasites from the rose aphid. Secondary parasitism has been as high as 70% on samples of 20 to 30 cocoons selected at random.

CONCLUSIONS.—Although no statistical data are included in this report, it is believed that sufficient evidence is presented to show why diapause in the rose-aphid parasites in Riverside, California, is extremely detrimental to the parasites' action and hence to the natural control of the rose aphid. This disadvantageous diapause exists in all four species under examination and in all probability results from poor synchronization or lack of adaptability to this aphid on this host plant.

Physical factors, aphid and plant physiology, and predators rather than parasites, appear to be responsible for the ultimate decline in the aphid population in late spring. The secondary parasites are quite detrimental to the effectiveness of the primary parasites. This fact is due primarily to the continuity of suitable cocoons during the entire season, and to the fact that these secondary para-

sites will attack various species of aphidiines apparently without showing any degree of specificity.

Perhaps the real explanation of this poor parasite-host relationship is simply that these parasites did not evolve biologically with this particular aphid.

It is thought that if one or more parasites having a diapause or life history more synchronized with the rose aphid's developmental history were introduced into the Riverside area, the present threat of rose-aphid damage could be greatly reduced in much the same way as has recently been done for the spotted alfalfa aphid in California (van den Bosch *et al.* 1959).

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The Effect of Phorate Applied to Seed on the Growth, Development and Insects Attacking Grain Sorghum[1]

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ABSTRACT

Seed of sorghum variety RS610 was treated with phorate (Thimet 44-D) at the rate of 0.5, 1.0, 2.0 and 4.0 pounds of active ingredient per 100 pounds of seed, with and without Arasan 75 at 10-2/3 ounces. Additional treatments included Arasan 75 and Sticker 25-1 alone and in combination. In field tests the 2- and 4-pound rates seriously reduced stand and delayed plant development as measured by pollen shedding. Plants from seed treated with the 0.5- and 1-pound rates shed pollen earlier than the untreated. Highly significant differences in seed yield were associated with differences in stand. The plots receiving the phorate treatments had significantly less yield than the untreated. In greenhouse tests, germination was reduced by the higher dosages and prolonged about 3 days. Treated seed, after 10 months storage, showed a great reduction in germination. Per cent germination under greenhouse conditions was highly significantly correlated with the field stand. Arasan reduced the toxic effect of the phorate. The sticker showed some deleterious effect on germination, which was reduced when combined with Arasan. The higher dosage rates controlled the corn leaf aphid, *Rhopalosiphum maidis* (Fitch), during the earlier period of growth. However, the phorate treatments gave no control of aphid populations on bagged heads of sorghum in the field.

Hurd-Karrer & Poos (1936) reported that wheat growing in soils containing sodium selenate was not attacked by mites and aphids. This finding and the introduction of the organic phosphate systemics as reported by Schrader (1948), developed a widespread interest in chemicals which can be safely introduced into plants and animals to control insects feeding on them. An excellent review of the literature of systemic insecticides has been given by Giang (1954).

A recent chemical which has shown systemic action as a seed treatment is *O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate now assigned the common name of phorate, and formerly designated Thimet. Although showing excellent control of certain insects when applied to the seed prior to planting, phorate has exhibited phytotoxic properties. Gojmerac (1956) reported that phorate in granular form at 1 and 3 pounds and at the 1-pound rate in activated carbon stunted sugar beet seedlings in greenhouse tests. Reynolds *et al.* (1957) reported that phorate applied to seed at the rate of 8 ounces was toxic to aphids on alfalfa for 5 to 6 weeks, when plants were growing rapidly. The material lost its effectiveness when plants were about 6 inches high. Granulated phorate applied in the row gave good initial kill of aphids and remained effective for a longer period of time. The 1-pound rate gave good control of leafhopper on sugar beets. On cotton, 1 pound controlled thrips for 4 to 5 weeks, aphids 5 to 6 weeks and reduced populations of the garden fleahopper. The southern garden leafhopper, *Empoasca solana* DeL., was reduced 66% 6 weeks after planting and flea beetle damage was reduced 76%. The 0.5-pound rate of phorate was almost equally effective in controlling the aphid but did reduce damage by the flea beetle. Phorate also showed promise in controlling whiteflies, leafminers, darkling beetles and black cutworms. Some marginal burning occurred on the cotyledons of newly emerged cotton, alfalfa and sugar beet seedlings. At the higher dosages burning was more pronounced but did not affect the true leaves. Seed treatments injured soybeans at the 1-pound rate, but granulated formulations were safer. Residues disappeared from the plants in 6 to 8 weeks.

Hacskaylo & Clarke (1957) reported that phorate applied to cotton seed did not delay germination and controlled aphids for 57 days after planting. However Dob-

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son (1958) found that phorate delayed blooming for a period of approximately 50 days after planting but produced no difference in height of plants. Yield was not affected. He also found that varieties responded differently. Parencia *et al.* (1957a, 1957b), in field tests found that phorate at the 1-pound rate did not delay germination except following heavy rains. Thrips were controlled for 5 weeks. However, periodic conventional spray schedules were more effective than seed treatments. Parencia *et al.* (1958) reported that cotton plants were injured when cotton seed was planted in drill rows previously planted with treated seed. Flowering and fruiting was delayed 4 weeks. These workers also reported that plants from reginned seed were more severely injured than those from flame- or acid-delinted seed. Dobson (1957) reported that phorate was ineffective in preventing damage by the spotted alfalfa aphid to seedling alfalfa. Bowling (1957) reported that phorate at 2, 4, and 8 ounces delayed emergence and stunted rice plants. It did not kill the rice water weevil or prevent oviposition.

Beckham & Morgan (1958) in extensive field tests on cotton found that phorate at the rate of 25 pounds per acre had no effect on stand. They reported early bloom was delayed but late bloom was unaffected. Thrips were reduced at all but one location. Aphids were controlled at all locations. Boll weevil was not affected. Late aphid counts showed reductions at only one location.

MATERIALS AND METHODS.—*Seed Treatments.*—Seed of sorghum variety RS610 was treated by the American Cyanamid Company in May 1958 with phorate (Thimet 44-D), a sticker (25-1) composed of 3.0 parts dextrin, 1.5 parts Sorbital and 0.5 parts water, and a fungicide Arasan 75. The various dosages and combinations of these materials are given in tables 1, 2, and 3.

Field Studies.—Treated sorghum seed was planted June 6 in six randomized blocks with four 10-foot rows per plot. The seed was planted with single row hand seeders at a heavy rate and later thinned after stand was established. On July 3 a visual rating of the stand in each plot was made. On August 26, the numbers of heads present in each plot was recorded and examined. The numbers of heads were classified as 0%, 25%, 50%, 75% and 100% of heads in bloom. At this time 10 heads from each plot were enclosed in large paper bags. These were removed September 29 and the degree of aphid infestation on the enclosed heads was recorded. On October 10 these heads were harvested to obtain seed yields.

Greenhouse Studies.—To obtain comparable data under more controlled conditions the treated sorghum seed was planted in 10-inch pots in the greenhouse July 18. Ten seeds were planted in each pot and the treatments were arranged in six replicated blocks. On July 25 the number of plants present in each pot was recorded. On August 29 the plants in the pots were examined for natural aphid infestation. A final stand count was made on September 12. At this time the height of each plant, the numbers of plants with heads, and the aphid infestation by plants recorded. In March 1959 the viability of the seed harvested

Table 1.—Effect of phorate applied to seed of sorghum variety RS610 on the stand and rate of germination in the field and under greenhouse conditions. Lafayette, Indiana. 1958, 1959.

Seed Treatments[a]				Greenhouse Studies				
				1958			1959	
Phorate (lb.)	Per Cent Sticker 25-1	Ounces Arasan 75	1958 Field Stand[b]	Stand (%) July 18	Stand (%) Sept. 12	Period of Germination[c] (Days)	Stand (%) Mar. 20	Period of Germination[c] (Days)
None	None	None	7.8	76.7	78.3	9.2	20.6	9.0
None	None	5.3	8.2	81.7	81.7	9.2	26.4	8.2
None	2	None	7.8	75.0	81.7	10.7	15.4	7.2
None	3	10.6	7.8	65.0	73.3	10.2	27.0	8.7
0.5	3	None	7.5	63.3	65.0	9.2	13.6	6.3
1.0	3	None	6.7	56.7	63.3	9.7	8.0	7.3
2.0	3	None	3.5	21.7	35.0	10.5	5.4	8.0
4.0	4	None	1.5	0.0	11.7	12.3	1.0	7.0
0.5	3	10.6	7.5	80.0	81.7	9.3	24.6	9.7
1.0	3	10.6	7.2	53.3	73.3	12.2	22.6	8.3
2.0	3	10.6	5.5	31.7	61.7	12.5	16.6	10.2
4.0	4	10.6	1.8	8.3	26.7	12.0	9.0	12.0
L.S.D. at 19:1			1.7	18.9	18.5	1.7	4.0	1.5
With Arasan			6.3	53.3	66.4	10.9	21.0	9.5
Without Arasan			5.8	48.9	55.8	10.3	10.7	7.4
L.S.D. at 19:1			.4	7.7	7.6	.7	1.6	.6
With phorate			5.1	39.4	52.3	11.0	12.6	8.6
Without phorate			7.9	74.6	78.8	9.8	22.4	8.6
L.S.D. at 19:1			.4	8.2	8.0	.7	1.8	.7

[a] Applied per 100 pounds of sorghum seed by the American Cyanamid Company in May 1958, Sticker 25-1 composed of 3.0 parts dextrin, 1.5 parts Sorbital and 0.5 parts water. Field planted—June 6. Greenhouse plantings—July 8, 1958 and March 7, 1959.

[b] Rating: 0—no plants to 9—perfect stand. Data taken July 3, 1958.

[c] From time of planting until final stand was complete.

Table 2.—Effect of phorate applied to seed of sorghum variety RS610 on the development of floral parts, growth and seed yields in the field and under greenhouse conditions. Lafayette, Indiana. 1958.

Seed Treatments[a]			Greenhouse Studies			Field Studies					
				Height (in.)					Yield per Head[c]		
Phorate (lb.)	Per Cent Sticker 25-1	Ounces Arasan 75	Plants with Heads (%)	Final	Adjusted for Stand	Plants with Heads (No.)	Pollen Shedding Heads (%)	Pollen Shedding Indices Rating[b]	Gross (gm.)	Adjusted for Stand (gm.)	Viability (%)
None	None	None	24.7	31.7	33.7	41.3	37.8	21.5	66.6	73.1	21.0
None	None	5.3	18.7	33.6	36.0	52.3	52.2	37.6	65.0	73.0	22.4
None	2	None	17.0	31.7	33.6	39.2	59.2	45.5	67.5	74.0	17.0
None	3	10.6	14.7	30.8	31.9	42.0	50.3	33.0	65.5	72.0	24.6
0.5	3	None	16.7	32.5	33.4	46.3	63.5	45.9	66.3	71.7	23.4
1.0	3	None	9.2	31.8	32.3	34.5	61.0	40.3	64.9	67.3	21.4
2.0	3	None	16.7	36.3	34.0	41.3	24.8	13.0	77.6	68.0	18.0
4.0	4	None	.7	39.6	35.6	22.8	12.2	7.5	91.3	74.1	21.6
0.5	3	10.6	6.0	31.7	34.0	50.8	50.8	33.4	67.9	73.3	25.4
1.0	3	10.6	9.8	32.4	32.5	41.8	55.0	38.7	61.7	65.9	20.6
2.0	3	10.6	4.2	32.0	30.5	40.3	35.7	24.5	72.2	70.0	22.6
4.0	4	10.6	0.0	37.1	33.7	10.5	9.5	3.3	88.1	72.1	17.6
L.S.D. at 19:1			n.s.	4.0	3.2	11.0	18.9	19.6	8.2	5.3	n.s.
With Arasan			8.9	32.9	33.1	46.4	40.6	28.4	70.1	70.4	22.2
Without Arasan			14.2	33.9	33.8	37.3	43.1	29.0	72.4	72.2	20.4
L.S.D. at 19:1			n.s.	1.6	3.2	4.5	7.7	8.0	3.3	5.3	n.s.
With phorate			7.9	33.3	34.4	36.1	39.1	25.8	73.8	74.8	21.4
Without phorate			18.8	31.9	31.5	43.7	49.9	34.4	66.1	64.4	21.2
L.S.D. at 19:1			n.s.	1.7	3.2	4.8	8.2	8.5	3.5	5.3	n.s.

[a] Applied per 100 pounds of sorghum seed by the American Canamid Company in May 1958. Sticker 25-1 composed of 3.0 parts dextrin, 1.5 parts Sorbital and 0.5 parts water. Planted in field June 6, 1958; in greenhouse July 8, 1958.

[b] Indices are based on numbers of heads showing 0%, 25%, 50%, 75%; and 100% pollen shedding. These averages are weighted by the total numbers of heads in each class.

[c] Taken from the 10 heads bagged to develop aphid infestations.

in the field in 1958 was tested. In addition, the 1958 remnant seed was planted in greenhouse flats. The treatments were planted in rows, 12 rows to a flat randomized in each of six flats. Records were kept daily of the numbers of plants present in each row until a final stand was obtained. When the plants were 25 days old, other plants of sorghum infested with corn leaf aphid were cut and laid between the rows in each flat. When these plants dried the aphids migrated to the growing plants resulting in a very uniform and good infestation of aphids. After 2 weeks these plants were dissected and the number of plants infested and the aphids per plant were recorded.

RESULTS.—*Germination and Stand.*—In the field tests it was evident that some treatments resulted in a severe reduction in stand. Since the number of seed planted per plot was unknown and subsequent thinning had been done, it was impossible to get a quantitative measure of the actual plant survival. This thinning to obtain uniform stand obscured the effects of the phytotoxicity of the lower dosage rates, whereas in those treatments where severe injury occurred little thinning was necessary. The data on stand and rate of germination are given in table 1.

The visual estimates of the stand in the different treatments indicated that the 2- and 4-pound rates of phorate reduced the stand highly significant amounts. A comparison of the average stand rating of all treatments involving Arasan with those not containing this fungicide, indicated that phytotoxicity was reduced significantly. When only the treatments involving phorate with and without Arasan were compared there was an increase of 0.7 in the rating where Arasan was included. The most significant reduction was due to the phorate which had an average stand rating of 5.1 compared with 7.9 in those plots where the insecticide was not used.

The percentage of plants surviving from seed planted in pots in the greenhouse July 8 was correlated with the field ratings of stand. The correlation coefficient was 0.975, highly significant. The high dosages of phorate reduced the germination in highly significant amounts. The presence of Arasan reduced the toxicity of the phorate 10.6% when all plots involving Arasan were considered. When only those treated with phorate were compared, the addition of Arasan increased the per cent survival from 43.8% to 60.8%. There was a reduction of 26.5% in stand in the plots containing phorate when compared with the treatments without the insecticide. When the remnant seed was planted in the greenhouse in March 1959, approximately 10 months after treatment, a severe reduction in germination was observed. This was general for all the treatments, but was more pronounced in the high dosages of phorate where Arasan was not included. Treatments with phorate alone showed an average reduction in viability of 85.5%. Those receiving Arasan in addition to the phorate, had a reduction of 69.8%. Where the sticker was used alone, there was a reduction of 81.1% in the seed viability. This might indicate that in storage

Table 3.—Effect of phorate applied to seed of sorghum variety RS610 on the populations of the corn leaf aphid developing in the field and under greenhouse conditions. Lafayette, Indiana. 1958, 1959.

Seed Treatments[a]				Greenhouse Studies				
				1958		1959[d]		
Phorate (lb.)	Per Cent Sticker 25-1	Ounces Arasan 75	1958 Field Infestations[b]	Pots Infested[c] (%)	Plants Infested Sept. 12 (rating)	Plants Infested Mar. 20 (%)	Aphids per Infested Plant	Aphids per Plant
None	None	None	4.2	33	1.0	80.3	26.7	23.8
None	None	5.3	4.0	17	1.5	66.0	17.0	15.2
None	2	None	3.3	50	1.9	65.8	18.0	10.1
None	3	10.6	3.6	50	1.6	82.8	29.0	26.6
0.5	3	None	3.1	83	1.4	55.5	12.2	9.1
1.0	3	None	3.6	50	1.7	42.0	4.8	3.6
2.0	3	None	4.5	0	1.1	22.2	.7	.6
4.0	4	None	5.4	0	.7	0.0	0.0	0.0
0.5	3	10.6	3.7	33	1.3	49.7	12.1	8.3
1.0	3	10.6	3.6	17	1.6	52.0	12.8	8.0
2.0	3	10.6	5.6	17	1.0	30.5	2.4	1.2
4.0	4	10.6	5.5	0	.6	27.7	3.1	1.8
L.S.D. at 19:1			1.7	42	.6	47.9	11.6	9.0
With Arasan			4.3	33	1.3	51.4	12.7	10.2
Without Arasan			4.3	25	1.3	44.3	10.4	8.1
L.S.D. at 19:1			.7	17	.2	11.7	5.7	5.0
With phorate			4.4	25	1.2	34.9	6.0	4.1
Without phorate			3.7	38	1.5	73.8	22.7	18.9
L.S.D. at 19:1			.7	18	.2	12.4	8.6	5.3

[a] Applied per 100 pounds of sorghum seed by the American Cyanamid Company in May 1958. Sticker 25-1 is composed of 3.0 parts dextrin, 1.5 parts Sorbital and 0.5 parts water. Planted in field June 6, in greenhouse July 8, 1958 and March 7, 1959.
[b] Taken from bagged heads, 10 per plot, rating: 0—no aphids to 9—head completely covered.
[c] Based on number of pots per treatment infested with aphids on August 19.
[d] Plots comprised single rows, 12 per greenhouse flat.

the sticker might be partially responsible for the reduction in germination. Although the treatment involving the sticker alone did show some lowering of viability when planted within a few months after treatment, it was not as pronounced as in the 1959 tests. A comparison of the treatments using phorate with and without Arasan showed an increase from 7.0% to 18.2% in seed viability when the Arasan was added to the treatment.

There was a highly significant linear correlation of 0.843 between the germination in the greenhouse studies in the July 1958 planting and the planting in March 1959. These data are shown in figure 1. It will be noted that the phorate treatments where Arasan was omitted all fell below or very close to the regression line. When Arasan was included, all the treatments were above the line. In addition, the treatment involving the sticker alone was highly significantly below the regression line. Those treatments involving Arasan alone and with the sticker were well above the line. This indicates the effective buffering action of Arasan in reducing the phytotoxicity of the phorate.

When the survival in the greenhouse in 1959 was compared with the field rating of stand in 1958, there was a highly significant linear correlation of 0.796. The relationship of the various treatments to the regression line followed an almost identical pattern as that for the germination in the greenhouse in 1958 and 1959. These data are shown in figure 2.

When the data for the germination in 1958 are compared with the field ratings for stand in 1958, as shown in figure 3, there is an almost identical pattern of relation-

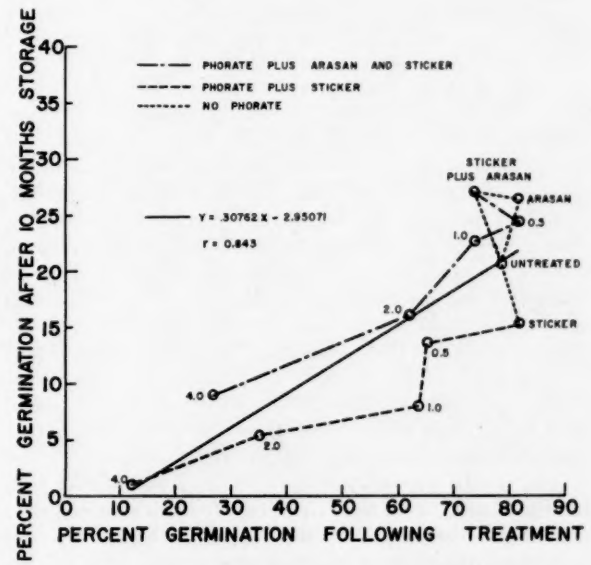

FIG. 1.—Phorate-treated sorghum seed—germination after 10 month's storage and immediately following treatment.

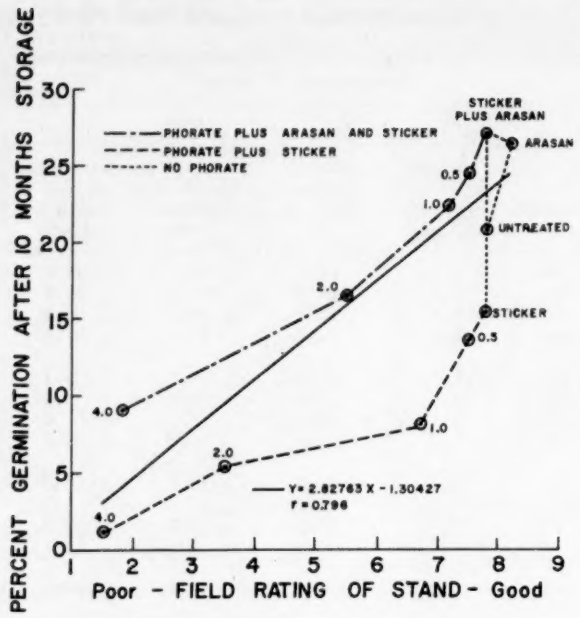

FIG. 2.—Phorate-treated sorghum seed—germination in greenhouse after 10 month's storage and field rating of stand.

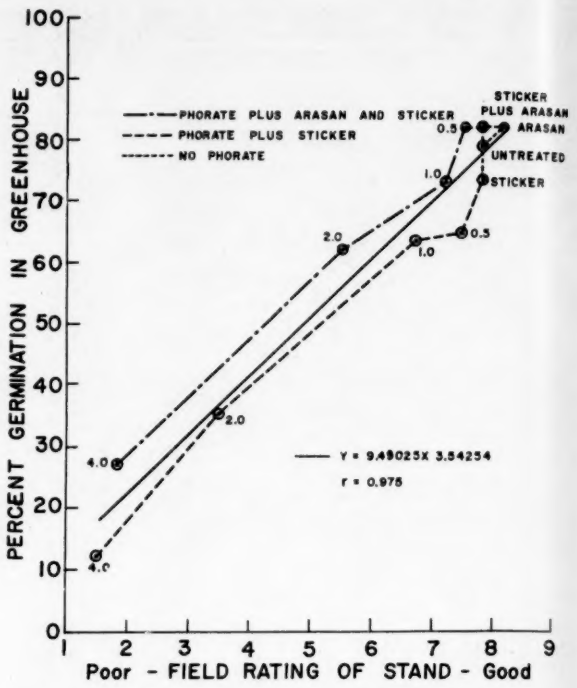

FIG. 3.—Phorate-treated sorghum seed—germination in greenhouse and field rating of stand.

ship of the various treatments to each other and to the regression line.

There were highly significant differences among the treatments in the July planting in the greenhouse in the length of time required for the plants from germinating seed to emerge from the soil. The longest period of time for emergence occurred in the high dosages of phorate and appeared more pronounced when the Arasan was included in the treatment. However, when the seed were planted 10 months after treatment, a much greater effect was noted, although the time interval was shorter, this may be explained by different cultural conditions.

Plant Development.—In observing the various field plots during the summer, it was obvious that there were differences in the numbers of plants headed on August 26 and the portion of the heads shedding pollen. The retardation in germination exhibited in the greenhouse by those treatments combining phorate with Arasan was reflected in numbers of plants with heads in these treatments in the field plots. Table 2 gives the data on plant development.

Pollen shedding was evaluated by a gross count of the heads present which were in bloom and by a weighted pollen shedding index, in which individual heads were examined and the number showing 0%, 25%, 50%, 75%, and 100% of the florets in bloom were recorded. The untreated plots and these receiving the 2- and 4-pound rates of phorate had significantly less bloom than the other treatments. The plants matured earlier in the 0.5-pound rate of phorate while those receiving the 4-pound rate were the most retarded. Only the plots treated with the 2-pound rate without Arasan and the 4-pound rate with and without Arasan were significantly more retarded than the other treatments. The plots treated with the 4-pound rate with Arasan were the only ones significantly less advanced than the untreated. When compared with the untreated plots, the treatments involving Arasan and the sticker alone and in combination with the two lower rates of phorate speeded up the maturity of the sorghum plants.

In the greenhouse tests the heads present on September 12 showed no significant differences. While the high variability of the data precludes drawing definite conclusions, the high dosages of phorate had the fewest heads present. Since similar effects were observed in the field tests, it may be assumed that dosages of phorate at the 2- and 4-pound rates delayed the development of the sorghum.

On September 12 all the plants surviving in the pots in the greenhouse were measured to the tip of the longest leaf or the head whichever was longer. There were significant differences in height among the various treatments. The 4-pound rate of phorate without Arasan and the 2- and 4-rate with Arasan were highly significantly taller than the other treatments. Since these treatments had significantly fewer plants surviving, less competition within pots might account for this increased growth. There was a highly significant linear correlation of 0.830 between the stand and the height of the plants. After the heights were adjusted for differences in stand, significant differences among the treatments still existed, although they were not entirely associated with dosages of phorate. The plants from seed treated with Arasan only were the tallest. The shortest plants occurred in the treatment where Arasan and the sticker were combined. Both treatments involving phorate at the 1-pound rate alone and at the 1- and 2-pound rates with Arasan were significantly shorter than those receiving only the Arasan treatment. The increased growth of the plants from seed treated with the higher dosages of phorate might be accounted for by the fact that the severe reduction in stand and resulting

lack of competition between plants occurring in these treatments more than compensated for the effect the phorate may have had on the growth of the plants.

Yields.—To estimate the relative yields, 10 heads were harvested from each field plot on October 10 and the weight of seed obtained (table 2). The yield per head increased in those plots receiving the high dosages of phorate. However, when these data were corrected for estimated stand per plot, the differences disappeared. There was a highly significant linear correlation of 0.957 between yield per head and estimated stand. Although there still remained significant differences in yield after adjusting for stand, these were associated with reduced yields in the plots treated with phorate at 1- and 2-pound rates withoug Arasan and the 1-pound rate with Arasan. In general the phorate treatment decreased sorghum yields significantly when compared with those treatments not receiving the insecticide.

Samples of the seed from each field plot, planted in greenhouse flats in the spring of 1959, averaged about 20% germination. There were no significant differences in the viability of the seed from the different treatments. Apparently the phytotoxic effects of the heavy dosages of phorate evident in the early development of the sorghum plants was of a transient nature and did not have any deleterious effect on the reproductive parts of the plants.

APHID INFESTATIONS.—The corn leaf aphid, *Rhopalosiphum maidis* (Fitch), is one of the more serious insect pests of grain sorghum. Infestations in the early stages of plant growth before the head appears are difficult to detect and control. The possible use of a seed treatment with a systemic insecticide to control this insect offers great advantages to sorghum growers.

Observation on aphid infestations of the sorghum plants grown from the seed treated with phorate, are given in table 3. In the greenhouse studies in the summer of 1958, natural infestation in the young plants were recorded by the numbers of pots in each treatment that were infested. There were significant reductions in the infestation in the 2- and 4-pound rate of phorate without Arasan and in the 1-, 2-, and 4-pound rates with Arasan.

At the time the sorghum plants were measured, the individual plants were rated for the numbers of aphids present. Although the infestation was light, there were indications that the phorate treatments at the higher dosages reduced the numbers of aphids present.

In March 1959, additional tests were made in which aphid infestations were induced by distributing infested sorghum among the rows of treated plants in the flats. Two weeks later the sorghum plants were dissected and the numbers of aphids present were recorded. In general the addition of phorate to the treatment reduced the infested plants more than 50%. The high rates of phorate without Arasan showed the greatest reduction in the number of infested plants. However, this was a rather severe test since the sorghum plants were in such close proximity that the leaves intermingled, and although the presence of aphids on the outer portion of the leaves were discounted, the aphids present in the whorl of the plant might have been recent introductions from adjoining plants and not yet subjected to the toxic action of the plant juices on which they feed.

The numbers of aphids per infested plant is an index of the ability of the aphids to develop on the plants in the various treatments. The 1-, 2- and 4-pound rates of phorate and the 2- and 4-pound rates with Arasan reduced the population highly significant amounts. In general the phorate treatment reduced the numbers of aphids present in the infested plants 75%.

An even greater effect of the phorate treatments is shown when the aphids per plant are examined. The 2- and 4-pound rate average 0.6 and 0 aphids per plant, respectively. When Arasan was added to the treatment these rates of phorate averaged 1.2 and 1.8 aphids per plant, respectively. The average of the 2- and 4-pound rates of phorate with and without Arasan reduced the aphid population approximately 96% when compared with the untreated plot.

One of the problems plant breeders of sorghum encounter is the tremendous buildup of corn leaf aphid populations in heads of sorghum covered with paper bags. To study the effect of the phorate treatments on the development of the aphid on the more mature plants, 10 heads in each plot were covered with paper bags. These were removed September 29 and estimates of the aphid population present were made. Although the variability of the data does not permit drawing conclusions, the heads from the seed receiving the highest dosages of phorate had the heaviest infestation. This would indicate that the application of phorate to sorghum seed at the rates tested was not effective in controlling this insect on the sorghum plants in the late summer.

CONCLUSIONS.—The results of these tests support the findings reported previously by earlier investigators. When used with a suitable fungicide the 1-pound rate of phorate had no phytotoxic effect on the sorghum seed. At 2.0 pounds per 100 pounds of seed there was a reduction of approximately 20% in germination. The 1-pound rate of phorate was more effective in controlling the corn leaf aphid when used alone than when applied with the fungicide. Since only a certain quantity of toxic material can adhere to a seed, this would indicate that either the addition of a fungicide reduced the amount of toxicant that could be applied to a seed or the presence of the fungicide reduced the absorption of the toxic material.

The reduction in toxicity to insects as the plants from treated seed grow is similar to the growth dilution effect observed in foliage applications. Since only a fixed quantity of toxic material adheres to a seed and is absorbed, as the plant grows larger this material is distributed throughout a greater amount of plant tissue. However, as the phorate is metabolized by plant processes after entering the plant, the resulting metabolites would similarly be limited in quantity and distribution, or further chemical changes may reduce their toxicity. Thus the toxic effect of systemic insecticides applied as seed treatments will be restricted in their effectiveness by the rate of plant growth and by chemical changes within the plant tissues to the control of those species of insects attacking the plants in their early stage of development.

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The Control of Poultry Lice and Mites with Several Organic Insecticides[1]

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ABSTRACT

In screening tests against three species of poultry lice, the shaft louse (*Menopon gallinae* (L.)), the chicken body louse (*Menacanthus stramineus* (Nitz.)), and the wing louse (*Lipeurus caponis* (L.)), and the northern fowl mite (*Ornithonyssus sylviarum* (C. & F.)), 0.025% to 1% sprays were applied to individual birds and the litter was treated with ¼ to 1 pound of 4% or 5% dust per 42 square feet. Co-Ral® (*O*-(3 chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) gave results comparable to malathion in spray tests at the 0.1% level. Sevin® (1-naphthyl-*N*-methyl carbamate) and dicapthon appeared to be the most efficient litter treatments.

Since the chlorinated hydrocarbon and organic-phosphorus insecticides have become available, new attention has been directed to the evaluation of materials and methods of controlling external parasites of poultry. Furman (1952) reported good control of the northern fowl mite (*Ornithnoyssus sylviarum* (C. & F.)) with 10% Neotran® (bis(*p*-chlorophenoxy)methane) dust but erratic data following single treatments of 0.5% to 2% sprays. Vincent *et al.* (1954), Furman & Weinmann (1956), Hoffman (1956), and Rodriquez & Riehl (1958) found 4% or 5% malathion dust and 0.5% to 3% sprays effective against both lice and mites either by direct application to the bird or indirectly through treatment of roost, house, or litter. Fairchild & Dahm (1955) found 1% to 5% dusts of several of the chlorinated hydrocarbons gave good control of lice when applied with a rotary duster at a rate of 15 gm. per bird or to the litter at 2 pounds per 100 square feet. Linkfield & Reid (1958) found 5% Dow ET-57 (now known as ronnel) and Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) and 10% Sulphenone® (*p*-chlorophenyl phenyl sulfone) promising against the northern fowl mite when applied with dust gun or in the litter. They also indicated 5% ronnel dust to be effective as a litter treatment against four species of lice.

At Stoneville, Mississippi, preliminary studies were made to determine the toxicity of several recent commercial and experimental insecticides to poultry lice and mites. The materials were applied directly to the birds as emulsion or wettable-powder sprays and/or as dusts mixed with the litter. The poultry had mixed, but not constant, infestations of the northern fowl mite, the shaft louse (*Menopon gallinae* (L.)), the chicken body louse (*Menacanthus stramineus* (Nitz.), and the wing louse (*Lipeurus caponis* (L.)). Most hens having fowl mites also had an infestation of lice, but many birds having lice did not show fowl mites.

Method of Rating Mite Populations.—Mite populations were rated on the basis of the average number observed per feather as follows: light, less than 1; moderate, 1 to 3; heavy, more than 3. Feathers were examined at the vent, neck, and both wings. The procedure was the same as reported in a previous paper (Hoffman 1956). All counting was done by the same person. Most of the birds had moderate to heavy infestations. These ratings and those given later for lice are presented only for the purpose of conveying the author's concept of population densities, as no standard schedule has been established by the various authors reporting on poultry parasites.

Lice populations were at least as prevalent on the skin as on the feathers and thus required a different technique of counting. The feathers were parted at two locations at the vent, breast, back, neck, and each wing and the total count was divided by 12, the number of partings. Populations were considered light when the average count was less than 3, medium when it was 3 to 7, and heavy when it exceeded 7. In general the population density was greatest near the vent, decreased farther forward, and was least on the wings. However, in severe infestations lice were found in medium to heavy numbers on all parts of the body. Data on birds having only the wing louse are not included in this report.

Most of the birds were white leghorn hens, but a few

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[2] In cooperation with the Delta Branch of the Mississippi Agricultural Experiment Station.

Table 1.—Spray tests for the control of poultry lice and mites.

Insecticide (Per Cent)	Pest	Per Cent Control After Indicated Days[a]						
		1	3	5	7	10	15	20
Dimethoate								
0.05	Lice	0	25	25	—	40	—	75
.1	Lice	50	50	70	—	60	—	60
.25	Lice	90	90	100	100	100	100	100
Dicapthon								
0.05	Lice	85	85	85	—	75	—	90
.1	Lice	50	85	50	—	50	—	100
.25	Lice	65	65	50	—	35	—	100
	Mites	35	65	90	90	65	—	—
.5	Lice	0	0	0	0	0	0	0
1.0	Lice	25	25	25	35	55	70	70
Malathion								
0.05	Lice	100	90	90	—	50	—	50
.1	Lice	65	75	85	100	100	100	100
	Mites	25	75	70	—	70	—	50
.25	Lice	95	98	100	100	100	100	100
	Mites	75	90	100	100	100	—	—
Delnav								
0.05	Lice	50	75	75	—	50	—	50
.1	Lice	75	90	90	—	95	—	100
.25	Lice	95	100	95	100	98	100	100
.5	Lice	85	85	100	100	100	100	100
	Mites	50	75	100	100	100	—	—
Co-Ral								
0.05	Lice	50	85	65	65	75	—	50
.1	Lice	70	95	100	100	100	100	100
.25	Mites	95	100	100	100	100	—	100
Ronnel								
0.05	Lice	—	50	—	75	—	—	75
.1	Lice	—	95	—	95	—	—	0
	Mites	—	50	—	50	—	—	0
.25	Lice	—	75	—	85	—	—	100
	Mites	—	0	—	0	—	—	0
.5	Lice	0	75	100	100	100	100	100
1.0	Lice	75	100	100	100	100	100	100
Sevin								
0.025	Lice	—	75	—	85	85	—	0
	Mites	—	50	—	90	90	—	50
.05	Lice	—	100	—	65	75	95	95
.1	Lice	40	50	70	65	75	95	95
	Mites	—	100	—	100	—	—	50
.25	Lice	50	100	100	100	98	100	100
Monsanto 10561[b]								
0.1	Lice	—	0	0	0	—	0	—
.25	Lice	—	0	0	0	—	—	—
	Mites	—	0	0	0	—	—	—
.5	Mites	—	15	40	15	—	—	—
1.0	Mites	100	100	100	100	100	—	—
	Lice	100	100	100	100	100	—	—
Monsanto 12432[c]								
0.25	Lice	0	0	25	—	—	—	—
.5	Lice	75	100	100	100	100	100	100
1.0	Lice	90	100	100	100	100	100	100
Trithion								
0.05	Lice	—	50	50	0	0	—	—
.1	Lice	—	50	50	0	0	0	—
.25	Lice	15	65	75	95	100	100	100
.5	Lice	65	100	100	100	100	100	100
Dowco 109[d]								
0.25	Lice	15	75	60	50	50	25	25
.5	Mites	0	75	85	100	100	100	100
1.0	Mites	0	100	100	100	100	100	100
Barthrin								
0.1	Lice	0	0	0	0	0	0	0
.25	Lice	0	0	0	0	0	0	0
.5	Lice	—	50	65	50	—	0	—
1.0	Lice	25	25	75	75	75	100	—

[a] Reported to the nearest multiple of 5, except when control exceeded 95% the exact figure is used.

[b] *s*-Tritbiane-*p*-chlorobenzenesulfenyl chloride-trimethyl phosphite reaction product.

[c] Diethyl 1-(dimethoxyphosphinyl)vinyl phosphate.

[d] Phosphoramidothioic acid, methyl-*O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl ester.

of the heavy breeds were also treated. With such breeds wetting of the feathers was more difficult and time to control was generally extended. However, no marked difference in time required for control was observed among the breeds when on litter. Birds on wire were subject to reinfestation from untreated birds in adjoining cages, whereas houses of birds on litter were separated sufficiently to make reinfestation more difficult.

Table 2.—Tests of four insecticides as dusts in litter treatments for control of poultry lice.

Insecticide	Pounds per 42 Sq. Ft.	Per Cent Control After Indicated Days					
		1	3	5	7	10	20
Malathion 5%	0.5	50	—	—	75	75	50
	1	—	100	—	100	100	100
Ronnel 5%	0.5	35	95	95	65	35	0
	1	—	100	—	100	100	100
Sevin 5%	0.5	100	100	100	100	100	—
	1	—	100	—	100	100	100
Dicapthon 4%	0.5	—	100	—	100	100	100
	0.25[a]	—	50	—	70	50	0

[a] Per 40 sq. ft.

Direct Application to Birds.—In these tests the insecticides were applied at the rate of 25 ml. per bird with a hand-operated continuous spray gun. During the spraying the birds were confined in a 12- by 12- by 8-inch cage made of 2-inch-mesh poultry wire, which was suspended and slowly rotated to give an even distribution of the spray. Ordinarily at any one date two birds were sprayed with the same concentration. Replicates were treated in the same manner on subsequent dates.

RESULTS.—The results of these tests are presented in table 1.

Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*- diethyl phosphorothioate) and malathion were the most consistent toxicants in controlling lice, and 0.1% emulsion eliminating all motile forms in 5 to 7 days in nearly all trials. Sevin® (1-naphthyl-*N*-methyl carbamate) and Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) at the same concentration also eliminated the original population in some trials, but usually after at least 7 days. In a few tests dimethoate gave high mortalities at 0.1% but was so erratic that the average control was low. 6-Bromo-piperonyl chrysanthemumate was ineffective against lice at all strengths between 0.1% and 1%.

Although fewer tests were made with mites, the same treatments were less effective than against lice. Malathion and Co-Ral were effective at 0.25%. Sevin at 0.1% provided control during the first week but did not eradicate the mites, whereas louse control was moderate during the first week but improved later. One bird treated at 0.25% was free of mites through 20 days' observation.

Litter Treatment.—Malathion, ronnel, Sevin, and dicapthon were applied as 4% or 5% dusts to the litter in small range-type houses so the birds might treat themselves as they fluffed their feathers. The litter was dry, crushed sugarcane stalk, a product the birds could readily stir. The dusts were distributed from glass jars having nail holes in their metal lids. Coverage was considered adequate at dosages from ¼ to 1 pound to 42 square feet

As shown in table 2, Sevin and dicapthon were effective at the ½-pound rate. Malathion and dicapton gave

control at 1 pound but were only partially effective at ½ pound.

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Effect of Virus Yellows on Yield and Quality of Sugar Beet Seed[1]

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ABSTRACT

Yield of sugar beet seed was reduced by virus yellows, curly top, and also from plants affected by both yellows and curly top. Germination of seeds from plants with yellows was reduced in one of four tests, but no reduction was attributable to curly top alone. Curly top and yellows together caused significant reductions in seed germination in two of three tests. In field plots, plants in various stages of development were inoculated with green peach aphids (*Myzus persicae* (Sulz.)) carrying the yellows virus. Reductions in yield occurred in all yellows plots; early infections caused greater reductions than later infections. Reductions in germination resulted from inoculations of plants in the bloom stage and in the early spring.

Little is known regarding the effect of virus yellows disease (also known as sugar beet yellows) on the production of sugar beet seed. Hull (1952), in England, found that plants infected early in their development may produce only 40% to 60% as much seed as virus-free plants, and the seeds may be smaller and lower in germination. Bonnemaison (1953) found similar reductions in yields under yellows conditions in France. McLean (1953) stated that, in the State of Washington, plants of table beets with virus yellows produced seed that were smaller and of lower vitality than healthy plants. Apparently no information has been published on the effects of this disease on production of sugar beet seed in the United States.

The most extensive beet-seed-producing area in the United States is in the Salt River Valley of Arizona, where seed is grown under contract for four large beet-sugar companies. All seed produced in this area is by the annual method—*i.e.*, planting in late August and harvesting the following June. Since 1955 yield and viability of the seed have been lower than at any time since the adoption of the lygus control program in 1945. Virus yellows was first observed in these fields in the spring of 1955. Studies were therefore undertaken to follow the development of virus yellows in the beet-seed crop.

Curly top is a problem in most areas in the West where beets are grown for sugar production, and it is no less a problem in the seed crop. Therefore, curly top was taken into consideration in planning the yellows studies. These studies were conducted in two ways: (1) tagging plants showing yellows or curly top, or both, in the field, and (2) inoculating plants artificially with virus yellows by the green peach aphid (*Myzus persicae* (Sulz.)) or with curly-top virus by the beet leafhopper (*Circulifer tenellus* (Baker)), or with both viruses. In both experiments the seed produced by the diseased plants was compared with seed from healthy plants. The tagged-plant studies were conducted on the crops harvested in 1956 and 1957, and the plot studies on the 1957 and 1958 crop.

In this work the chief criteria of damage were yield and per cent germination of large seeds, but since some indication was evident that diseased plants produced a larger percentage of small seeds than healthy plants the large and small seeds were separated by screening. Seeds more than 8/64 inch in diameter were considered large and those between 7/64 and 8/64 inch, small. Those less than 7/64 inch were discarded as trash. The small seeds, by the present standards, are not marketable.

TAGGED PLANTS.—Multigerm varieties of beets were used, and plants in fields of curly-top-susceptible beets were marked when they were in the bloom to soft-seed stage.

In 1956 plants were selected in groups of 3 and replicated 15 times, within each group 1 plant showing definite yellows symptoms, 1 both yellows and curly top, and 1 apparently healthy. Some of the plants were not found at harvest, but sufficient were recovered to make 10 replicate groups. The results, summarized in table 1, indicate that yellows in combination with curly top decreased the yield and germination and increased the percentage of small seeds. Yellows alone had a similar tendency, but the differences were not significant.

In 1957 plants in two fields were tagged in groups of 4, replicated 25 times, 1 plant in each group showing yellows, 1 curly top, 1 both curly top and yellows, and 1 apparently healthy. In a third field 25 groups of 2 plants each were selected, 1 showing yellows and the other apparently healthy. In 1 field 23 groups were recovered, in another 22, and in the third only 10. Data from seed harvested from plants in these fields are summarized in table 1. In field 1 there was a significant reduction in seed yield only for curly top; increases in the percentage of small seed were not significant, nor were the differences

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[2] Entomology Research Division, Agricultural Research Service, U.S.D.A.
[3] Crops Research Division, Agricultural Research Service, U.S.D.A.
[4] Western Seed Production Corporation.

FIG. 1.—Beet-seed plots inoculated with yellows virus as they appeared 36 days before harvest. The plants were smallest and seed set was lightest in plots A and B. There were no apparent differences between plots C, D, and E and the uninoculated plots (X).

in the percentage of germinating seed. In field 2 significant reductions in yield were caused by both diseases, alone and together. Increases in the percentage of small seed were indicated in some groups, but they were not significant. Significant reductions in germination were shown only in seed from plants infected with both yellows and curly top. Data for field 3 indicate reductions in yield and seed germination owing to yellows and corresponding increases in the percentage of small seed.

INOCULATED PLANTS.—In 1956 and 1957 yellows-infective aphids were placed on the plants without cages, allowed to remain 24 to 48 hours, and then killed with insecticides. Beet leafhoppers were caged on the plants in leaf-clip cages and allowed to remain several days. The results were inconclusive and are not presented, but they indicated that date of infection had a direct bearing bearing on the amount of damage caused by the yellows.

Beginning in the fall of 1957 successive inoculations were made with yellows until the plants were through the bloom stage the following spring. A curly-top-susceptible variety of sugar beet known as American 3S was used. The plots were arranged in a 6 by 6 latin square. Each plot consisted of two rows of beets 50 feet long with a two-row barrier strip between plots and a 10-foot alley at each end. Figure 1 shows these plots on May 6, 36 days before harvest. The plants were in the vegetative stage when the first two inoculations were made but by March 26 seed stalks had started to develop. On April 8 the plants were in the flower-bud stage and on April 21 they were in full bloom. It was approximately 7 weeks after the fall inoculations before yellows symptoms were noted, but only 3 to 4 weeks after the spring inoculations.

The data in table 2 show a significant decrease in yield of large seed for all dates of inoculation. The earliest inoculations gave the greatest reduction. The reductions represent losses ranging from 1,497 pounds per acre for the November 20 inoculations to 310 pounds for the April 21 inoculation. Reductions in yield of large seed were accompanied by increases in the percentage of small seed, but differences owing to treatment were not so consistent as for large seed.

The average weight of 100 large seeds was significantly lower from all infected plots than from noninoculated plots, but there were no differences between dates of inoculation.

There were highly significant reductions in germination

Table 1.—Effects of yellows, alone and in combination with curly top, on yield and germination of seed from sugar-beet plants tagged in the field, 1956 and 1957.

Treatment	Grams of Seed per Plant	Per Cent of Small Seeds	Per Cent Germination of Large Seeds
Experiments in 1956			
Yellows	50	30	51
Yellows and curly top	32	51	21
Disease-free	77	14	76
L.S.D. at 5% level	33	18	33
Experiments in 1957			
Field 1 (22 replicates)			
Yellows	28	27	86
Curly top	21	36	71
Yellows and curly top	27	33	70
Disease-free	31	22	82
L.S.D. at 5% level	6	—[a]	—[a]
Field 2 (10 replicates)			
Yellows	23	20	62
Curly top	18	40	57
Yellows and curly top	13	38	29
Disease-free	41	18	71
L.S.D. at 5% level	16	—[a]	16
Field 3 (23 replicates)			
Yellows	29	31	74
Disease-free	37	21	82
L.S.D. at 5% level	8	8	4

[a] Not significant by the F test.

of large seeds from plots inoculated April 21, when the plots were in the bloom stage. These reductions may have been caused by a more severe, or "shock," effect caused by the primary invasion of the flowering parts when they were in a critical stage of seed formation. The only other reduction in germination was found in plots inoculated February 21, at which time spring growth was well started. This reduction is difficult to explain, since neither the fall inoculation nor those on March 26 and April 8 had any apparent effect on germination.

Table 2.—Yield and quality of seed from sugar-beet plants inoculated with yellows virus on different dates, 1957–58.

Plot	Date of Inoculation	Large Seed: Grams per Plot	Large Seed: Pounds per Acre (Calc.)	Large Seed: Grams per 100 Seeds	Large Seed: Per Cent Germination	Per Cent Yield of Small Seed
A	Nov. 20, 1957	4,829	2,782	1.71	87.6	2.7
B	Feb. 21, 1958	5,385	3,103	1.60	84.3	2.8
C	Mar. 26, 1958	6,096	3,512	1.65	87.6	2.3
D	Apr. 8, 1958	6,407	3,692	1.71	88.7	2.2
E	Apr. 21, 1958	6,889	3,969	1.67	81.4	2.1
X	Noninoculated	7,427	4,279	1.91	89.8	1.7
L.S.D. at 5% level		321	185	0.13	3.0	0.4
at 1% level		438	252	0.18	4.1	0.6

No differences occurred in the weight or in germination of small seed owing to treatment. The germination ranged from 30% to 35%, which is too low for the seeds to be of commercial value and represents a loss to the grower.

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Evaluation of Insecticides for Control of the Fuller Rose Beetle on Citrus in California[1]

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Abstract

The Fuller rose beetle, *Pantomorus godmani* (Crotch), though not generally a serious pest of citrus, is found in groves in almost all the citrus-growing areas in California. Occasionally beetle populations become of economic importance, particularly on young trees. Field tests with insecticides known to be toxic to this beetle indicated a problem in timing and in persistence of residues, since chlordane, aldrin, endrin, dieldrin, toxaphene, and cryolite eliminated populations of Fuller rose beetle for only part of the year. Studies on the evaluation of the relative persistence of residues for the control of this beetle were conducted in the greenhouse. Dieldrin and Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3,-benzotriazine-3-methyl) phosphorodithioate) at all three dosage levels (1, 2, and 4 ounces of actual toxicant per 100 gallons of water) were the most effective materials tested.

The Fuller rose beetle, *Pantomorus godmani* (Crotch), has been reported from many parts of the world and on a large number of plant hosts, including citrus. This beetle is found in all the commercial citrus-growing areas of California, but injury to citrus groves is generally not serious. Damage to young trees is usually more serious, but occasionally populations become of economic importance on mature trees (Dickson 1950). Adult feeding on leaves is the most easily recognized type of injury, but root feeding by the larvae can also be severe on both young and old trees.

The Fuller rose beetle apparently has but one generation a year: The eggs are laid on the plants, and the larvae enter the ground and feed on roots until the next summer. They then pupate and emerge from July through November, with the peak emergence in September.

In addition to the life history studies briefly reviewed above, Dickson (1950) reported on the control methods available at that time.

The purpose of the present study was to evaluate the effectiveness of some of the new insecticides for the control of this beetle on citrus in California.

Methods and Materials.—Densities of adult beetle populations are relatively difficult to evaluate on citrus

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trees, owing to their protective coloration and lack of movement during the day. Attempts to evaluate adult beetle populations quantitatively by shaking the trees and dislodging the beetles onto ground cloths, and by searching specific tree areas such as lower foliage and inside branches failed to yield consistent samples. A 2-minute search per tree was finally adopted as the best method for determining beetle population densities.

A navel orange grove that was being seriously defoliated by adult beetles was used to determine the effectiveness of endrin, aldrin, dieldrin, chlordane, toxaphene, and cryolite. The trees in the various plots were thoroughly sprayed from ground level to a height of approximately 6 feet. The spray was applied at a rate of 200 to 300 gallons per acre by conventional high-pressure equipment with manually operated spray guns.

The relative persistence of toxic residues from a number of new insecticides was evaluated in the greenhouse by means of laboratory techniques. Citrus seedlings were thoroughly sprayed in a chamber with 1, 2, and 4 ounces of actual toxicant per 100 gallons. The materials tested are listed in table 1. Each insecticide spray was applied to two plants and allowed to dry at room temperature. Copper screen cages were then placed around these potted plants, and five adult beetles collected from field populations were confined on each plant. At weekly intervals the plants were examined, and when all the beetles were dead they were removed and replaced with five live beetles. This was repeated until none of the exposed insects were killed by the residues. The results obtained from three replications of each insecticide evaluated were averaged and expressed as minimum and maximum number of weeks during which the spray residue was toxic to adult beetles.

Table 1.—Duration of toxicity of several wettable-powder (WP) insecticides to the Fuller rose beetle on small citrus trees in the greenhouse.

Material and Formulation	Actual Toxicant/100 Gallons of Spray (Oz.)		
	4	2	1
Dieldrin, 50% WP	9–10 weeks[a]	9–10 weeks	7–8 weeks
Guthion, 25% WP	8–9	7–8	6–7
Malathion, 25% WP	7–8	2–3	<1
Chlordane, 50% WP	6–7	7	4
Chlorthion, 25% WP	4–5	2–5	3–4
Cryolite, 90% WP	4–5	—	—
DDT, 50% WP	4–5	4–5	4
Endrin, 50% WP	4–5	4–5	3–4
Parathion, 25% WP	3–4	2–3	3–4
Sevin®,[b] 50% WP	2–3	2	<1
Trithion®,[c] 25% WP	2	1–2	<1
Diazinon®,[d] 25% WP	<1	<1	0
Dipterex®,[e] 50% WP	<1	<1	0
Kelthane®,[f] 18.5% WP	0	0	0
Chlorobenzilate®,[g] 25% WP	0	0	0
Phorate[h], 25% WP	0	0	0
Untreated check	0	0	0

[a] Weeks after treatment until introduced beetles successfully survived residues.
[b] 1-naphthyl-*N*-methyl carbamate.
[c] *S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate.
[d] *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate.
[e] *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate.
[f] 1,1-bis(*p*-chlorophenyl)2,2,2-trichloroethanol.
[g] Ethyl 4,4′-dichlorobenzilate.
[h] Formerly designated as Thimet (*O,O*-diethyl *S*-(ethylthio)-methyl phosphorodithioate).

Table 2.—Relative effectiveness of several insecticides for the control of Fuller rose beetle on Valencia orange trees in California.[a]

Material and Formulation[b]	Amount Toxicant per 100 Gallons Water	Average Number Live Fuller Rose Beetles per Unit[c]			
		Pretreatment	11-5-54	12-9-54	8-3-55
Chlordane, 40% WP	3 lbs.	34.1	0	0	14.1
Aldrin, 40% WP	½ lb.	42.3	0	0	11.6
Aldrin, 40% WP	2 lbs.	38.7	0	0	12.9
Endrin, 19.5% EC	2 pts.	21.6	0	0	14.2
Dieldrin, 50% WP	1 lb.	16.9	0	0	13.4
Toxaphene, 40% WP	1 lb.	27.4	0	0	27.3
Cryolite, 90% WP	3 lbs.	23.5	0	0	19.1
Untreated check	—	18.3	17.9	17.2	12.6

[a] Treated October 15, 1954.
[b] Applied with conventional sprayer at the rate of 200 to 300 gallons per acre (thorough coverage from ground to 6 feet high on tree). WP = wettable powder; EC = emulsifiable concentrate.
[c] Unit: one tree searched for a 2-minute period.

RESULTS.—All the materials used in field tests (table 2) successfully reduced the adult beetle population on the trees for the remainder of the year. The appearance of many beetles the following year indicated that numerous eggs had hatched and that the larvae had entered the ground prior to treatment and therefore were not killed by the spray materials.

Control of this beetle over a period of more than 1 year might be obtained in various ways: (1) by timing applications of insecticides so that the adults would be killed upon emergence and before the eggs were laid, (2) by multiple applications of sprays having a short period of effectiveness for killing this beetle, and (3) by the use of a single application of an insecticide having long-lasting residual toxicity.

Additional field tests have not been possible to date because of the absence of sufficiently heavy populations of the beetles in California citrus groves. These light populations are probably the result of extensive commercial use of many of the insecticides shown in table 1 for control of other pests on citrus.

The results of laboratory tests (table 1) give a good indication of the relative length of time these insecticides protect the foliage from feeding by adult Fuller rose beetles. Dieldrin and Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) at all three dosage levels were the most effective materials tested. Dieldrin at only 1 ounce of actual toxicant per 100 gallons of water eliminated Fuller rose beetle from 7 to 8 weeks in the greenhouse, a longer period of time than any of the other insecticides tested except Guthion and chlordane at the 2-ounce dosage and malathion at 4 ounces. DDT, endrin, Chlorthion®, (*O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate) and cryolite at 4 ounces per 100 gallons were effective from 4 to 5 weeks. The rest of the materials had little or no effect in reducing the number of adults.

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SCIENTIFIC NOTES

Biology and Control of the Apple Seed Chalcid in North Carolina[1]

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The apple seed chalcid (*Torymus druparum* Boh.) has been recorded in crab apple (*Malis* sp.) and small seedling apples (*Malus sylvestrus*) (Cushman 1916). However, the first record of it in North Carolina was in the fall of 1953 when, Turnipseed & Mitchell (1955) found it most prevalent at higher elevations of the apple-growing area.

Cushman (1916) and Richardson & Allen (1953) found that some of the insects spend two winters in the larval stage.

Destruction of all crab apple and seedling apple trees, plus destruction of all culled fruit for two seasons were recommended for control (Cushman 1916). Richardson & Allen (1953) obtained control with one application of 0.75 pound of DDT per 100 gallons of water applied June 18 in Ontario.

A study of the life and seasonal history was begun in North Carolina in 1955 and continued through the spring of 1958. In the fall of 1955, several bushels of small Golden Delicious and Red Winesap apples were collected in Mitchell County and brought to the laboratory in Wilkes County. Seeds were removed from many apples, and those infested were detected by sight and touch as described by Turnipseed & Mitchell (1955). Two hundred infested seeds were placed in a pint jar, the mouth of which was covered with two layers of cheesecloth held in place with a rubber band. The jar was placed on the window sill between the window and screen on the north side of the laboratory. Emergence of adults was recorded through the spring of 1958.

Several jars of whole apples were placed beside the jar of infested seed and held until the spring of 1956. Then, 200 infested seeds were removed from the apples which were not completely decayed. These seeds were held through the spring of 1958 and the number of adults emerging from them each spring was recorded.

The remaining apples collected in 1955 were placed in emergence cages outside the laboratory for additional adult emergence records.

The larvae of the apple seed chalcid overwintered in the seed and pupated in late April. The adults emerged during a 10- to 14-day period in early May. They began egg laying soon after emerging, usually within 2 days. The first adults appeared when the immature apples were no more than one-half inch in diameter and completed emergence when apples were about 1 inch in diameter.

Some larvae spent two winters in the seed. None were found alive after three winters (table 1). The proportion of larvae spending the second winter in the seed may be influenced by the speed of breakdown or decay of the apple. When the infested seeds were removed from the apples in 1955, 96.8% of the adults emerged in the spring of 1956 and 3.2% of the adults emerged during the second season. No emergence occurred after two seasons. When the seeds were not removed from the infested apples until early spring of 1956 and the apples came through the winter with little rot or breakdown, 80% of the adults emerged in 1956 and 20% emerged in 1957. No emergence occurred from these seeds after two seasons (table 1).

Table 1.—Emergence of apple seed chalcid from seed removed from apples in North Carolina.

Year	No. Infested Seed 1955	No. of Adults Emerging 1956	1957	1958	No. Failing to Emerge
1955	200	150	5	0	45
1956[a]	200	100	25	0	75

[a] Records made from apples not completely decayed.

Table 2.—Results of insecticide test against apple seed chalcid, Spruce Pine, North Carolina. 1957.

Treatment[a]	Application Date	Small Apples Infested (%)
DDT 2 lbs. 50% W.P.	May 21	0.0
	May 21 and June 4	4.0
Malathion 4 lbs. 25% W.P.	May 21	0.0
	May 21 and June 4	4.0
Demeton 1 qt. 26.2% E.C.	May 21	28.0
Thimet 1 qt. 47% E.C.	May 21	38.0
Delnav 1 qt. 50% E.C.	May 21	41.3
Sevin 2 lbs. 50% W.P.	May 21	1.3
	May 21 and June 4	1.3
Check (3 lbs. lead arsenate)	Throughout season	46.6
L.S.D. at 5% level		12.6
at 1% level		17.3

[a] All treatments with 100 gallons of water.

An experiment for control of this pest was set up in 1957 in a block of Golden Delicious trees. Each treatment was applied to one-tree replicates, replicated three times and randomized throughout the block. The first application was on May 21, 10 days after petal fall or at first cover, when apples were one-half inch in diameter. The second application was on June 4, 2 weeks later, or at second cover, when the apples were 1 to 1½ inches in diameter. One and two applications were made with DDT, malathion, and Sevin® (1-naphthyl-*N*-methyl carbamate). One application was made with demeton, Thimet® (*O,O*-diethyl *S*-(ethylthio) methyl phosphorodithioate), and Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate). The above treatments were in addition to the regular lead arsenate program applied by the grower throughout the season.

Data on control are presented in table 2. One application of each of the following materials gave effective control: DDT, malathion, and Sevin. Two applications did not increase effectiveness. Demeton, Thimet, and Delnav were relatively ineffective.

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The Effect of Insecticides on Transamination in the American Cockroach[1]

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It was observed that the injection of the amino acids glutamate and aspartate into the hemocoel of the American cockroach, *Periplaneta americana* (L.), caused tremors and paralysis lasting

Table 1.—The effects of insecticides on transamination in *Periplaneta*.

In Vivo		*In Vitro*	
Test	Changes in Optical Density per 10 Minutes[a]	Test	Changes in Optical Density per 10 Minutes
Water	0.041	Acetone	0.031
DDT	.040	DDT	0
Toxaphene	.043	Toxaphene	0
Endrin	.045	Endrin	0
Malathion	.092	Malathion	0
Sodium cyanide	.008	Sodium cyanide	.005
Sodium orthoarsenate	.047	Sodium orthoarsenate	.035
		Water	.038

[a] The values given are the means of three determinations.

for several hours. Larger amounts of glutamate (0.1 ml. of M solution) caused tremors, paralysis, reduction of the rate of heart beat, and death after about 24 hours. The same quantities of the analogous keto acids, oxaloacetate and α-ketoglutarate, caused no visible symptoms. The toxicity of many amino acids (but not glutamate and aspartate) when administered in excess to vertebrates was reviewed by Kamin & Handler (1957).

These results suggested that the effects of insecticides on enzymes catalyzing amino acid metabolism in insects might be important. Therefore, the effects of certain insecticides on the glutamate-aspartate transamination reaction were studied. Enzymic activity was followed spectrophotometrically after the method of Green *et al.* (1945) by noting the increase in optical density at 280 millimicrons of a reaction mixture containing aspartate, α-ketoglutarate, borate buffer, and fat body homogenate.

The insecticides, namely aqueous solutions of 1% sodium cyanide and 1% sodium orthoarsenate and acetone solutions of 5% DDT (Green Cross), 5% malathion (Niagara Spray), 5% toxaphene (Hercules Powder) and 2% endrin (Shell Oil) were tested both *in vitro* and *in vivo*. In the *in vitro* method, 0.1 ml. of the aqueous solution of an inorganic insecticide or 0.01 ml. of an organic insecticide was added to the transaminase reaction mixture consisting of 0.5 ml. of 0.1 M aspartate, 0.5 ml. of 0.1 M α-ketoglutarate, 3.9 ml. of borate buffer at pH 8.3, and 0.1 ml. of fat body homogenate. In the *in vivo* method, cockroaches were killed with large amounts of the insecticides, control tests consisting of cockroaches drowned in water; their fat bodies were dissected out and homogenized in 10 times their weight of water, and were assayed for glutamate-aspartate transaminase activity using the transaminase reaction mixture described above.

The results of both methods are given in table 1. In the tests *in vitro* all the insecticides except sodium orthoarsenate inhibited enzymic activity, while *in vivo* the organic insecticides had no effect, only sodium cyanide inhibiting. Malathion appeared to increase enzymic activity.

It was concluded that only sodium cyanide inhibits the transaminase enzyme *in vivo*, and it is suggested that the *in vitro* inhibition by the organic insecticides was an artifact due to the adsorption of protein enzyme by the resulting suspension of insecticide. A similar suggestion was made by Brown & Brown (1956) in studying the inhibition of cytochrome oxidase, and the mechanism of *in vitro* inhibition is thoroughly discussed in that paper. The effects of the water-soluble inorganic insecticides agree with the results discussed by Cohen (1951).

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Control of the Oriental Rat Flea with Systemic Insecticides Fed to Rats[1]

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In areas where rat fleas may be vectors of organisms causing human diseases, control of fleas prior to controlling rats is desirable to reduce the danger of spreading disease when fleas abandon their dead hosts. DDT has been used with success in rat runways to control fleas on rats (Davis 1945). However, it appeared that fleas might be controlled on rats by systemic insecticides. The effectiveness against cattle grubs, *Hypoderma lineatum* (De Vill.) and/or *H. bovis* (L.) of the three systemics used in this study were reported by Lindquist (1956), Hewitt *et al.* (1958), and Brethour *et al.* (1958). A systemic insecticide appears promising if it controls fleas and is acceptable to rats with an anticoagulant rodenticide such as warfarin. Since several days of successive feeding on anticoagulant poisons are required before rats die, this could provide an opportunity for fleas to be poisoned by feeding on rats that consumed the systemic insecticide.

The purpose of this investigation was to determine whether the oriental rat flea, *Xenopsylla cheopis* (Rothsch.), was killed when exposed to white Norway rats, *Rattus norvegicus* (Erxleben) given oral treatments of ronnel, Dowco 109 (Phosphoramidothioic acid, methyl (*O*-(4-tert-butyl-2-chlorophenyl) *O*-methyl ester) or dimethoate, formerly designated Am. Cyanamid 12,880 (*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate).

METHODS.—Fleas were reared on white rats according to the technique described by Smith & Eddy (1954). The insecticides ronnel, Dowco 109, and dimethoate were administered orally in 1 ml. of 10% alcohol either as emulsions or solutions. The rates of treatments were 25, 200, and 300 mg./kg. for dimethoate, Dowco 109; and ronnel, respectively. Three white rats were treated with each insecticide, and three rats used for controls were drenched with 1 ml. of 10% alcohol. Immediately after the treatments, 15 newly emerged fleas were confined in a clear plastic container to a 1-square-inch area on the back of each rat. Dead fleas were removed from each rat and counted daily for 3 days. Each rat had access to water and dry dog food during the test.

In another experiment two rats were treated with dimethoate using the same method and rate described above, and two untreated rats served as controls. Immediately after treatment each rat was confined to a 5-gallon can containing 1 pint of dry sand and 400 newly emerged fleas. Live fleas were removed from the rats and sand and counted 3 days after treatment.

RESULTS.—Control of fleas caged on rats given oral treatments of systemic insecticides is shown in table 1. Dimethoate controlled fleas at the end of 1 day significantly better than did

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Table 1.—Average cumulative numbers of dead fleas 1, 2 and 3 days after caging 15 fleas on each of three rats per treatment.

Treatment	Rate (mg./kg.)	Average Cumulative No. of Dead Fleas per Rat, on Days Following Treatment		
		1	2	3
Dimethoate	25	12.3	12.7	12.7
Dowco 109	200	2.0	6.3	8.3
Ronnel	300	2.3	6.0	8.0
Check		1.0	2.0	2.3
L.S.D. at 5% level		3.9	6.5	5.6

Dowco 109 or ronnel. Two days after treatment dimethoate controlled significantly better than ronnel and approached significantly better than Dowco 109. Ronnel and Dowco 109 appeared to give some control but were not significantly different from the check 1 and 2 days after treatment. Differences between treatments, considering total dead fleas for the 3-day period, were not significant, but treated rats had significantly more dead fleas than untreated rats.

Two rats treated with 25 mg./kg. of dimethoate and each exposed to 400 fleas had 52 and 82 live fleas 3 days after treatment compared with 361 and 293 live fleas recovered from two untreated rats. Under these conditions dimethoate appeared to give about 80% control.

Discussion.—Although some flea control was achieved in these tests, it is not known whether systemic insecticides would give better or poorer control under natural conditions. Since control probably depends on feeding by the fleas, poorer control could be expected in the field if newly emerged fleas, used in this experiment, feed more than fleas feed under natural conditions. On the other hand, fleas subjected to the abnormal conditions of these tests may feed less than normal. Wild Norway rats at Hays, Kansas, had a sufficiently low population of oriental rat fleas that it seemed impractical to evaluate insecticides with them.

According to Davis (1945) and Morlan & Hines (1951) DDT was generally less effective for control of the spined rat louse (*Polyplax spinulosa* (Burm.)) and the tropical rat mite (*Ornithonyssus bacoti* (Hirst)) than of oriental rat fleas. Lice and mites were not included in these tests; however, systemic insecticides may provide better control of rat lice and mites than of fleas, since lice and mites remain on the host more nearly continuously.

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Control of *Myocoptes musculinus*[1] on Guinea Pigs[2]

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During an experiment to study the effects of microwave energy (Addington *et al.* 1958), several guinea pigs became infested with mange. It was necessary to control the causative agent as soon as possible because this disease would make it extremely difficult to determine accurately the effects of the radiation dosages.

Myocoptes musculinus (Koch 1844), the myocoptic mange mite, was identified from material taken from suppurating lesions on several of the small mammals. Myocoptic mange in laboratory mice was reported to have been eradicated with DMC also designated Dimite® (4,4′-dichloro-*alpha*-methylbenzhydrol) (Stoner & Hale 1953) and excellent control was obtained with Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chlorethyl sulfite) (Flynn 1955). However, the effects of DMC and Aramite on guinea pigs were unknown (Baker *et al.* 1956). Therefore, the following treatments were undertaken with caution.

Both experimental and control guinea pigs, were divided into two lots. The first group was submerged in a 50% ethyl alcohol solution of 0.2% DMC, and the other was dipped in a 2% concentration of Aramite plus a liquid detergent as a wetting agent. This was done once a week for 3 weeks. In both cases the open lesions began healing after the first application and the animals began to gain weight. At the end of the third week the coats had returned and the animals were apparently normal.

Only one animal died as a probable result of the treatment. This guinea pig was in very poor condition owing to radiation and extensive mange and went into convulsions after the first application of Aramite. Other than this one case no differences were noted between the effectiveness of DMC and Aramite. Both acaricides provided satisfactory results.

To maintain control it was also necessary to disinfect the shelves and floors of the cages systematically with a lysol solution and to sterilize all other equipment. The guinea pigs were maintained free of mites for at least 8 months after the three treatments and sanitary precautions.

No guinea pigs under 2 months old were used, so the effects on very young animals are not known.

Results of this experiment indicate that it is safe and effective to use the prescribed dosages of DMC and Aramite for the control of *Myocoptes musculinus* on guinea pigs in the laboratory.

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[1] Acarina, Listrophoridae.

[2] Accepted for publication September 1, 1959.

Experiments on Prolongation of the Life of Mosquito Larvae by Underfeeding[1]

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Mosquito larvae are excellent subjects for testing the toxicity of insecticides (Marcovitch 1928). Various media may be employed for growing the larvae, such as dried blood, blood albumin, and yeast, or dog biscuits.

With such good diets, the larvae of *Aedes aegyptii* (L.) are able to reach the pupal stage within 5 days or even less. According to Bates (1948) "The normal food of most mosquito larvae seems to be microorganisms, particularly bacteria, yeasts, and protozoa, as well as any detritus that may be small enough to be picked up by the action of the mouth brushes in filtering the water." The accessory growth factors, such as thiamin and riboflavin, required by mosquito larvae for normal development, are ordinarily supplied by living microorganisms. A third factor of unknown chemical nature, but present in a "flavin-purine complex" is also necessary according to Trager (1937).

Wigglesworth (1929) kept a predaceous mosquito larva of the genus *Megarhinus* alive 5 months at 24° C. by limiting its food supply. The food of such predatory larvae would naturally supply all the needed nutritional elements.

The question arose as to the nutritional value of such incomplete materials as sugar, starch, and bolted corn meal when used alone as compared with milk alone. The eggs of *Aedes aegyptii* were supplied by Insects Affecting Man, Research Laboratory, Entomology Research Division, Orlando, Florida. These eggs were placed in vials 4 inches by ½ inch, half filled with unsterilized tap water, and kept at room temperature. On skim milk powder the larvae grew very well. After reaching the third instar they were underfed and remained alive 104 days, when they were accidentally destroyed.

With tap water alone, the larvae hatching from the eggs lived 13 days. On gelatin alone the larvae lived 30 days. On bolted corn meal and underfeeding, some of the larvae were kept alive 121 days. On cornstarch only, the larvae reached the third instar and remained alive 120 days. On 0.5% sucrose, some of the larvae became nearly full grown and remained alive 133 days.

The interesting point in these experiments is the growth and survival on such incomplete foods as starch and sugar. In the case of the cornstarch there is the possibility of the existence of a small percentage of protein. Sucrose, however, is pure carbohydrate. A slight fermentation may have supplied some of the necessary vitamins, but what was the source of the protein? Are we to surmise the existence of a small amount of nitrates or nitrogenous matter in the tap water that could have been used for protein synthesis? Perhaps some of the protein was furnished by the bacteria, living and dead, that are known to be present in potable tap water even after chlorination and "purification."

Underfeeding is known to prolong life. By alternately feeding and underfeeding Child (1935) was able to keep earthworms alive and active for 3 years, but when well fed they pass through their life cycle in 3 weeks.

With higher animals, such as rats, Carlson and Hoelzel (1946) increased the life span by intermittent fasting of one day in three. Rats kept underweight by restriction of calories outlived those allowed to fatten (McCay *et al.* 1941).

We thus have examples from various kinds of animals of the prolongation of life through underfeeding.

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A Systemic Insecticide and Bait for Flea and Rat Control[1]

ELWIN BENNINGTON[2]

It is standing procedure in a rodent-born disease-control program for ectoparasite control to precede rat-killing. This procedure at present necessitates two programs, insecticide dusting followed by rodent control. A substantial economy would result if an effective systemic insecticide could be incorporated into attractive rat baits that would kill vector fleas before the rodent hosts are killed.

Systemic insecticides have been used for the control of many insect pests (Drummond 1958, McGregor & Bushland 1956). One of the more successful of these has been ronnel (Trolene[®][3]) which is used against cattle grubs, *Hypoderma* spp. (Anonymous 1958), Knapp *et al.* 1958a), the sheep bot (Peterson *et al.* 1958), sheep blow flies (Marquardt & Hawkins 1958), demodectic mange mites (Sanger 1958), and fly larvae (Knapp *et al.* 1958b). The formulation used here, 10% of ronnel emulsion concentrate on soybean meal for administration in feeds, has a very definite but not objectionable taste. It was mixed into rat baits to determine its palatability to wild Norway rats (*Rattus norvegicus*) and to determine if the acceptable levels were effective for the control of rat fleas.

MATERIALS AND METHODS.—Previous work (Hayes & Gaines 1950) indicates that average-size adult Norway rats consume approximately an ounce of dry food per day and that if baits are attractive and well placed, it is presumed they would consume up to 15 grams of bait per day. To ingest 5, 10, 20, 50, or 100 mg. insecticide per kg. of body weight an adult rat must consume at least 20, 40, 80, 200, or 500 mg. of insecticide mixture, respectively, per 15 gm. of bait. Mixed into 1-pound lots of coarse-ground whole yellow corn (Hayes & Gaines 1959) the desired levels of insecticide required 0.6, 1.2:2.4, 6.0, 12, 24 and 48 grams, respectively, of the 10% ronnel-on-soybean meal formulation per lot. Each lot was identified by a number indicating the insecticide content.

To test palatability the baits were placed in a large warehouse containing waste grain, animal feed and a large population of Norway rats. The baits were placed in four groups with plain cornmeal and mixtures containing various levels of ronnel in each group to offer the rodents a free choice of baits. The anticoagulant, fumarin, was added to the ronnel baits at the recommended dosage of 0.025% and the new mixtures were grouped as before in the same warehouse. These baits were exposed for 2 weeks and replenished three times a week.

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[2] United States Department of Health, Education, and Welfare, Public Health Service, Bureau of State Services, Communicable Disease Center, Atlanta, Georgia.

[3] The Trolene used for this investigation was supplied by the Dow Chemical Company, Midland, Michigan.

Table 1.—Records of rat-feeding on baits containing ronnel, December 31, 1958.[a]

Field Group	Ronnel per Pound of Bait (Gm.)				
	0	2.4	6	12	24
I	+++	+++	+++	++	++
II	+++	+++	+++	+++	++
III	+++	+++	++	+++	++
IV	+++	+++	+++	+	++

[a] + bait visited, small amount of it eaten.
++ much bait was eaten.
+++ bait eaten completely.

To determine the effectiveness of the insecticide levels accepted by wild rats the bait mixtures (Hayes & Gaines 1959) were fed to laboratory rats. Large (300+ gm.) rats were placed in pairs in small insect-proof boxes that were constructed to prevent insecticide from the bait (food) being carried back into the nest chamber and acting as a contact poison. Oriental rat fleas, *Xenopsylla cheopis* (Rothsch.), were placed in the nest chambers at the beginning of each test. At least one pair of rats in each test was fed cornmeal without insecticide.

Results.—A large wild Norway rat population, estimated by numerous droppings of various sizes, active runways and numerous rats observed, took insecticide levels up to 2.4 gm. per pound as readily as plain cornmeal (table 1). The 6-gm.-level baits were taken well, the 12-gm. level was taken in some areas and not in others while the higher levels were definitely avoided.

The ronnel-fumarin baits were taken with results substantially the same as those obtained without fumarin. No additional aversion attributable to the fumarin was observed. These baits were exposed and visited for a 2-week period when rats were observed to be poisoned and were reported by workmen to be "sick."

When given free choice between plain cornmeal and cornmeal-ronnel mixtures laboratory rats showed aversion to the insecticide-bait mixture. After 3 days, all the cornmeal was eaten and only half of the 2.4-gm. ronnel-meal. In another trial only small amounts of 6-gm. ronnel-meal were taken.

When given no alternative, the ronnel-meal was taken very well. While many fleas survived at the 2.4-gm. level, they were killed effectively at the 6-gm. level (table 2).

An anticoagulant poison was incorporated into the insecticide-cornmeal to determine whether fleas could be killed before the rats began to die. A 6-gm. ronnel-cornmeal-fumarin mixture killed all test fleas by the third day while 135/150 fleas survived in the control box. All the rats were dead after 5 days.

The 6-gm. bait was very effective for captive rats but wild rats could be induced to take only one third to one half of their daily diet as an acceptable bait. When the 6-gm. ronnel-fumarin bait was diluted with two parts of plain cornmeal, 66/150 fleas survived in the test box.

Since the 12-gm. bait was avoided to some extent by wild rats its attractiveness was enhanced by adding 10% sugar and 20 ml./lb. of a commercial liquid-smoke condiment. To prolong the survival time for rats the fumarin was reduced to a 1:49 dosage. This mixture when offered to four rats with plain cornmeal as an alternative was very palatable, the rats taking after 5 days exposure three quarters of the cornmeal and one half of the bait mixture.

Table 2.—Survival of fleas on rats[a] being fed ronnel baits.

Ronnel per Pound of Bait (Gm.)	Test Days	Survival of Fleas	
		In Test	Control
2.4	6	134/150	—
6.	4	1/150	106/150
6.	4	0/150	81/100
12.	3	0/250	145/250

[a] Four rats used in each test.

The 12-gm. ronnel-smoke sugar-fumarin bait was diluted with two parts of plain cornmeal and given to rats with 250 fleas in each box. The test fleas were dead after 3 days, the rats showed symptoms of poisoning on the fourth day and died after 5 days. Flea survival in a repeat test was 0/250 and 3/250.

All of the ronnel-bait mixtures resisted infestation by grain-infesting insects while some of the insecticide-free baits were infested with the red flour beetle, *Tribolium castaneum* (Hbst.). American cockroaches (*Periplaneta americana* (L.)) and oriental cockroaches (*Blatta orientalis* (L.)) were allowed to feed on the 12 gm. ronnel bait and all died after 2 days.

Discussion.—Rodent control is desirable to reduce danger of murine typhus in the Southeastern States, to reduce a potential danger of plague in the Western States and to reduce annoyance due to rat bites and rat fleas in substandard housing areas. Whenever permanent bait stations are established for rat control, the use of an insecticidal bait has an additional advantage in that it kills vectors as well as reservoir hosts.

A 10% sugar, 1:49 fumarin-cornmeal containing 12 gm. ronnel and 20 ml. liquid smoke per pound was attractive to rats, killed oriental rat fleas before the rats died in a feeding period of 5 or more days and resisted stored food pests.

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Action of DDT at an Insect Synapse[1]

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In spite of its economic and historical importance, the primary site and detailed mechanism of action of DDT remain obscure. From the general symptoms of intoxication a primary ac-

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tion on the nervous system is likely. Tobias & Kollros (1946) and Roeder & Weiant (1948) concluded that peripheral sensory structures were most sensitive, although Dresden (1948) contradicts this. All of these investigators and also Sternburg & Kearns (1952) have reported no effect on the central nervous system. Nevertheless, Dresden (1949) proposed that the synapse might be the site of lethal action.

The work of Hoyle (1952) indicates that the neurilemma in insects is an important barrier between the neuromuscular tissues and the hemolymph. It seemed possible that this sheath might hinder the penetration of DDT to sensitive regions in the central nervous system, thus accounting for the negative results reported from relatively short term electrophysiological experiments. This paper reports the effect of application of DDT to a desheathed cockroach ganglion.

METHOD.—The preparation chosen was the last abdominal ganglion of the American cockroach, *Periplaneta americana* (L.), in which a synaptic relationship was worked out by Pumphrey & Rawdon-Smith (1937). Abdomens were removed from young adult male cockroaches and the nerve cords exposed from the dorsal aspect. All peripheral nerves except the cercal were cut. The ganglion was desheathed as described by Twarog & Roeder (1956), except that only a partial desheathing was done in order to minimize damage to the ganglion. Pointed silver wire stimulating electrodes were placed on a cercal nerve or in the cerci and similar pickup electrodes on the ascending connectives. A Grass S-4C stimulator, P-5 preamplifier and Dumont 304A oscilloscope were used.

Test solutions were made up by serial dilution in Ringer-Hoyle solution. Since DDT is quite insoluble in water (less than 0.1 p.p.m.) and effective concentrations are higher than this, it was necessary to work with fine suspensions. These were made by first dissolving the DDT in minimal amounts of hot ethanol and then injecting this into saline. Control solutions had the same amount of ethanol in them as the DDT suspensions with which they were compared.

RESULTS.—Cercal nerves were stimulated 10 times a second with 0.2 msec. pulses of sufficient intensity to bring in just one giant fiber with single responses in the ascending connective. When DDT at a suspension strength equivalent to a 10^{-5} M solution was added, the post-synaptic fiber responded with multiple spikes within a few seconds to 2 or 3 minutes (fig. 1). At first the response changed to two spikes per stimulus, then three, four, and eventually more or less continuous firing. Within a train of impulses successive spikes came at longer intervals. Reducing the stimulus intensity produced no response. However, DDT application usually brought in no additional giant fibers; it was necessary to increase the stimulus intensity to activate these. Appropriate controls containing approximately 10^{-4} w/v ethanol were not active on these preparations.

In the initial stages when doubling or tripling of the response occurred it was sometimes possible to restore the single response by rinsing with saline. Later, the effect was not reversible and progressed to a long continuous train of spikes which eventually slowed to a stop. The fiber was then inexcitable by increased cercal stimulation.

These effects were not found on ganglia with the sheath intact, or on those prepared from the ventral aspect.

DISCUSSION.—Since the effect was found only in preparations where the ganglion had been partially desheathed, this action of DDT must be central, synaptic, rather than on the cercus or cercal nerve. The action appears similar to that reported by Roeder & Weiant (1948) on proprioceptors and by Smyth & Roys (1955) on chemoreceptors where response to stimuli was converted by DDT from single spikes or short trains to long trains of action potentials. It might also be compared with the effect reported by Gordon & Welsh (1948) which led them to propose that their DDT-trains originated in "pacemaker" regions of crayfish nerves. Receptors, pacemakers and synapses are places where local excitatory processes predominate. Possibly DDT acts to prolong (or delay recovery from) local excitation. Thus adjacent conducting regions would be induced to develop extra action potentials when their refractory periods have passed.

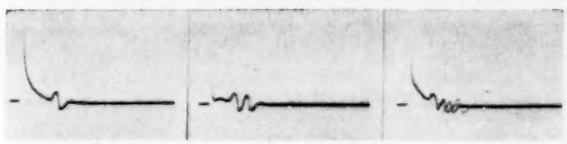

FIG. 1.—Multiple response after application of DDT to desheathed ganglion. Left to right: before, 20 seconds after, 1 minute after treatment.

It has been possible, at least partially, to dissociate symptoms, including knockdown, from lethal action. For example, Hodgson & Smyth (1955) could produce symptoms by local DDT treatment and then abolish them by excising the treated area. On the other hand DDT seems just as lethal when treated insects are kept under continuous carbon dioxide anesthesia and thus prevented from showing any symptoms at all. Peripheral action should be more rapid owing to normal means of encounter with the insecticide and greater sensitivity there, and could account for most of the overt behavioral symptoms. Central action, with an extra barrier to pass, should take longer and could lead to death.

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The House Cricket, *Acheta domesticus*, and the House Fly, *Musca domestica*, as a Model Predator-Prey System[1]

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A laboratory model predator-prey system is available in the association of the house cricket, *Acheta domesticus* (L.), and the

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house fly, *Musca domestica* L. The advantages of this particular system for studying the mechanisms which underlie the dynamic relationship of predators and prey are explained.

Both the house cricket and house fly have relatively short life cycles and are easily maintained in the laboratory. At 80° F. the cricket has a life cycle of 90 days, while that of the house fly is about 12 days. Both species tolerate a wide variety of conditions, allowing the populations to be reared together successfully in many different environmental situations.

Experimental studies of density-dependent interaction are easily achieved by combining older nymphs and adult crickets with house fly pupae in population cages. The older nymphs and adult crickets consume house fly pypae. Both predation responses may be demonstrated. The functional response, (Solomon 1949) where predation pressure varies according to prey density, has the basic form of Holling's (1959) type II. Before satiation the number of fly pupae consumed is directly proportional to the number of prey. Following the start of satiation, there is an inverse relationship between the number of prey consumed with an increase prey density, finally approaching an asymptotic level. Comments on the characteristics of the numerical response (Slomon 1949), where predation pressure varies according to predator density, are not possible at this time.

When an experimental design necessitates the continued survival of the predator and prey species together, rearing procedures may be modified to accomplish this. For such experiments, a population cage, consisting of a transparent plastic dish, 12 inches by 9½ inches by 4½ inches, was employed. Four 1-inch diameter holes were made in the lid and covered with 60-mesh brass screening to provide ventilation. Dry sand, ¼ inch deep, was placed on the bottom of the cage. Added wood excelsior provided moulting and resting places for the crickets. A small glass "baby food" jar was filled with water and inverted on a cellucotton-filled petri dish to provide water in each cage. Two smaller plastic boxes, 2⅞ inches square by 1½ inches high, were filled with damp sand and placed in each cage to provide an oviposition site for the crickets. The sand was kept moist to allow for suitable egg development. Cricket eggs which were maintained under these conditions at a temperature of about 80° F. hatched after 17 days.

In preliminary tests, it was found that the young crickets in the first two thirds of nymphal development were unable to eat house fly pupae but did survive on chick mash. When the chick mash was placed in a small dish, 2 inches in diameter by ¾ inches deep, and screened by a cover of ½ inch square hardware cloth, only the young crickets would secure the chick mash.

House flies can be husbanded in the population cages for the whole or a part of their life cycles. House flies may be reared separately from the cricket population and only the pupae introduced into the cages. Using this method, the investigator can determine the prey density.

To maintain house flies throughout their life cycle with the crickets, various stages of house flies are first introduced into the population cages. Vials (1 inch diameter by 4 inches high) containing cellucotton soaked in yeast and milk and water mixture are inserted in the cages to provide the adult flies with food and an oviposition site. The fly larvae also live in the milk-water and yeast mixture in the cellucotton. The mixture is made up by adding one part canned milk to two parts water to which is added 0.001 part cooking yeast. At the completion of the larval period, they crawl out of the vials to pupate in the sand which is in the bottom of the cages. By changing the depth of the dry sand in the bottom of the cages, a protective sanctuary of varying effectiveness is provided. Those pupae found are eaten by the crickets. Seldom will a cricket attack a squirming larva. In such a system, the variable responses of cricket and fly populations may be studied.

A test population consisting of 15 adult crickets, 30 in the last third of nymphal development, 20 in the middle third, and 100 in the first third, consumes about 100 house fly pupae per day. The smaller the pupae, the more easily they are eaten and the greater number consumed. Pupae of desirable size can be obtained by appropriate sieving.

Crickets find pupae by contacting them directly with their antennae and the maxillary palps. When pupae are covered with sand about $\frac{1}{32}$ inch deep, the crickets cannot find them, although at this depth the pupae are clearly visible to the human eye. If by chance the cricket kicks the thin layer of sand and exposes a part of the pupa, it is usually found and eaten. When the density of the pupae located in one area was increased, there was no proportional increase in the frequency with which the pupae were found. Thus, it is suspected that odor has little or nothing to do with finding pupae at any distance.

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Laboratory Method for Screening Compounds as Attractants to Gypsy Moth Males[1]

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This is a preliminary report on the use of behavioral observations in the laboratory to detect substances that may be attractive to male gypsy moths (*Porthetria dispar* (L.)) in nature.

The method used was a modification of a technique of Minnich (1922). Male moths, collected in the fie'd or reared from field-collected pupae or laboratory-reared, were gently grasped by their bodies and secured by the central portions of their wings to plastic, spring-type clothespin mounts, which were then hung from pins on a wooden rack. A period of acclimatization was allowed which usually eliminated the flight response induced by the absence of contact of the legs with a substrate.

During tests, compounds on pieces of filter paper, on cartridges made of filter paper, on glass rods, or in vials were held 1 to 2 cm. from the distal portions of the antennae. Each insect was exposed to a compound for not more than 1 minute. Tests were made at least 15 minutes apart to compensate for the effects of adaptation and fatigue. The tests were conducted in still air, but air was circulated in the laboratory between tests. The temperature was maintained at 77° F.

Natural and synthetic substances were tested with the clothespin technique. A 1956 extract of the abdominal tips of gypsy moth females collected in the U.S.A. served as a control. The criteria of comparison were the relative time taken to induce the copulatory response, the relative intensity of the reaction, and the number of insects responding.

When the control extract was presented to a mounted male that was quiet and sexually receptive, the first activity usually consisted of flicking movements of both antennae, followed by rapid vibrations of the wings and spreading movements of the legs. Then the abdomen usually bent in the direction of the extract and remained in a curved position after cessation of stimulation. Wing vibrations also usually continued after the extract was removed. Sometimes, this entire copulatory behavior was observed to occur almost at one time.

[1] Accepted for publication September 28, 1959.

[2] Present address: Lycoming College, Williamsport, Penna.

[3] Field-collected pupae and adults were supplied by Edward Paszek of the Plant Pest Control Division and his field survey crew; laboratory-reared stock was made available by Franklin Lewis of the Northeastern Forest Experiment Station; and most of the test materials together with results obtained with them in the field were provided by Martin Jacobson of the Entomology Research Division.

A cartridge impregnated with a 1956 extract of the abdominal tips of Spanish gypsy moth females and field-tested with negative results during the 1958 season was also inactive on laboratory insects, but another cartridge of the same extract, not previously field-tested, was effective.

Benzene, acetone, and one of the synthetics, octyl caprylate, that attracted several males in 6 days of field testing during 1958, usually elicited the copulatory behavior pattern when exposed to test moths. However, the initial response was different from that given to the natural material; the insect repeatedly flicked its prothoracic legs toward the stimulus. This behavior is attributed to the irritating properties of such materials and may lead to spurious results unless the initial movements are closely observed.

A commercial formulation, the active ingredient of which is 1,2-epoxyhexadecane, that was very active during field testing in 1958 was also attractive to field-collected males but its activity remained uncertain when tested on laboratory-reared moths because their sexual responses were very erratic.

From these preliminary tests the laboratory results appear in general agreement with field data obtained on the attractiveness of the various compounds. However, since various irritating chemicals consistently elicited sexual behavior, caution should be exercised when interpreting the responses of the insects to active compounds. Also, since tests on laboratory-reared moths proved more inconsistent than those on field-collected material, the general physiological condition of the insects must be considered as an important experimental variable.

Finally, the routine use of this screening technique is based on two assumptions: (1) Compounds that in their highest concentrations do not elicit responses from male moths in the laboratory will not be attractive in nature, and (2) compounds that in their highest concentrations elicit copulatory responses from male moths in the laboratory will also be attractive in the field. The validity of these assumptions awaits further testing.

In previous summers the various fractions of the sex extract, as well as many synthetics and botanicals, could be field-tested only during the moths' brief flight period. Given a laboratory supply of moths, the clothespin technique, even with its limitations, should permit testing for a longer time with less expense, effort, and starting material.

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Membracidae[1] Attracted to Black Light[2]

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Reports on significant numbers of Membracidae responding to light are scarce. In Pennsylvania, Frost (1955, 1957) reported on unprecedented numbers of treehoppers collected in Minnesota-type light traps, equipped with a 60-watt tungsten filament lamp. His largest collection for one night was 6,289 specimens, estimated on the basis of a dry weight sample.

In Wisconsin, a large flight of Membracidae occurred after dusk on June 6, 1959, in a residential area of Middleton, Dane County. The insects were observed first at window screens and around house lights. Many terminated flight on clothing, in the hair of people, and on automobiles. As this activity had not been noticed previously, the intensity and duration of flights was investigated further.

Two black light traps were placed on the ground in the yard of a residence. One trap had two 15-watt BL lamps; the other had one 6-watt BL lamp. The traps had been fabricated by the Agricultural Engineering Research Branch, U. S. Department of Agriculture, and were experimental models of omnidirectional design with the lamps mounted vertically in the center of a single baffle. The traps were located 80 feet apart and separated by a corner of the residence. In general, the residential area had been developed in a mixed-oak wood lot within the past 10 years and contained many oak trees 40 to 50 feet tall.

Table 1.—Numbers of Membracidae and certain other insects caught at black light traps, Middleton, Wisconsin.

	Date and Trap				
	June 7	June 8		June 9	
	Both	6-Watt	15-Watt	6-Watt	15-Watt
Membracidae[a]	58,065	6,799[b]	68,906	24,071	32,524
Corixidae	755	88	154	40	56
Trichoptera	1,220	216	1,737	76	112
Lepidoptera	490	186	426	316	351
Coleoptera	360	76	307	83	168
Temperature °F. during trapping period					
Minimum	66	66		63	
Maximum	82	82		78	

[a] The membracids were measured in a 100-ml. graduate. Two 100-ml. samples gave 3625 and 3728 specimens, respectively. The average (3675) was used to estimate the numbers of membracids.

[b] The trap's funnel became plugged and many specimens piled up on the ground. These were not collected, but would have increased this number.

Table 2.—Species of Membracidae collected in black light traps, Middleton, Wisconsin; with annotations on previous light trap records.

Cyrtolobus arcuatus (Emmons)
C. auroreus Woodruff [Frost '55]
C. discoidalis (Emmons) [Dennis '52]
C. fuliginosus (Emmons) [Dennis '52]
C. fuscipennis Van Duzee
C. helena Woodruff [Dennis '52]
C. maculifrontis (Emmons)
C. pallidifrontis (Emmons) [Frost '57]
C. querci (Fitch) [Frost '55, '57]
C. tuberosus (Fairmaire)
C. vau (Say) [Slingerland '02, Funkhouser '17, Dennis '52, Frost '55, '57]
Ophiderma evelyna Woodruff [Dennis '52]
O. flava Goding
O. grisea Woodruff [Dennis '52]
O. pubescens (Emmons) [Funkhouser '17, Dennis '52, Frost '55]
O. salamandra Fairmaire [Dennis '52]
Smilia camelus (Fabricius) [Dennis '52, Frost, '57]

Previous records at lights—other species:
Slingerland, 1902 [*Cyrtolobus castanea* Van Duzee, *Cerasa bubalus* Fitch]
Funkhouser, 1917 [*Cyrtolobus castanea* Van Duzee, *Campylenchia latipes* Say]
Dennis, 1952 *Palonica tremulata* (Ball)
Frost, 1955, 1957 [*Cyrtolobus fenestratus* (Fitch), *Ophiderma flavicephala* Goding].

The traps were operated from 8:00 P.M. to 6:30 A.M. (CST) during the period June 7 through June 12. On the first night the traps were observed from about 10 P.M. to midnight. The immediate area surrounding each trap was "swarming" with Membracidae. Many individuals coming to the traps seemed to settle around them. In the morning, Membracidae inactive but living were piled on the ground beside the traps, especially around the 6-watt BL trap. It was estimated that the pile around the smaller trap contained about half the volume of its catch. Similar results were experienced on the following three nights, when there also was considerable flight activity.

[1] Homoptera.

[2] Accepted for publication October 8, 1959.

The numbers of Membracidae caught in the traps greatly exceeded other common insect groups (table 1). The catches began to subside on June 11 (14,884 membracids) following a cooling trend at midnight and a minimum temperature of 61° F. The flight stopped almost entirely on June 12 (204 membracids) when the minimum temperature dropped to 53° F. Homoptera other than Membracidae were not caught in the traps during the period except for a few typhlocybine leafhoppers. Data for the night of June 10 are not given, but catches were comparable to those of June 9.

Seventeen species in three genera were identified[3] among the more than 200,000 specimens collected in the traps (table 2). Five species are recorded at lights for the first time. The collections consisted almost entirely of males. The very few females that were found included *Cyrtolobus fuliginosus* (Emmons), *C. fuscipennis* V. D., *C. querci* (Fitch), *Ophiderma evelyna* Woodruff and *Smilia camelus* (Fab.).

This note confirms Frost's record that large numbers of males of certain oak-inhibiting Membracidae are responsive to lights when the lights are located close to infestations. Also, our observations suggest that suitably located black light traps would be useful for studying the flight activities of these insects.

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[3] The authors express their sincere appreciation to Dr. C. J. Dennis, East Central State College, Ada, Oklahoma, for his helpful determination of the species.

Experiments for the Control of Sap Beetles in Sweet Corn Grown for Processing[1]

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During the past few years sap beetles, primarily *Carpophilus lugubris* (Murr.), have become more serious pests of sweet corn grown for processing, and a greater need for efficient, economical control of these insects has arisen. Sap beetle adults, varying in numbers, can be found in corn fields during the entire time that the ears are developing. Oviposition takes place in the ears throughout this period (Harrison & Ditman 1958). The larvae hatching from eggs deposited in ears cause the serious sap beetle injury to the corn kernels and create a contamination hazard in the processed product. The nature of the injury and infestation is such that trimming is difficult and expensive. A narrow margin of profit in corn grown for processing limits the amount of chemical control that is economically feasible.

That the DDT-oil emulsion treatments usually recommended for control of the corn earworm (*Helioths zea* (Boddie)) are not effective against sap beetles has been demonstrated by Connell (1956), Ditman *et al.* (1955), and Harrison & Ditman (in press). However, Connell (1956) has shown that a number of organic phosphorus insecticides are somewhat effective against sap beetles. Whitlaw *et al.* (1959) demonstrated the effectiveness of airplane applications of malathion, parathion and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate). Because of the need for a less expensive, yet effective control, experiments were conducted in order to evaluate the efficacy of the addition of organic phosphorus insecticides to the recommended DDT-oil emulsion sprays for control of the corn earworm.

Procedure.—In 1958, a small plot experiment of the randomized block design was conducted using several organic phosphorus insecticides: parathion, malathion, Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), Diazinon and dimethoate. The materials were applied with a high clearance corn sprayer. Each plot was treated three times except for the plots receiving dimethoate which were sprayed only once on August 3. The treatments on the 4 remaining plots were begun on July 28 when 10% to 20% of the plants were in silk. Succeeding applications were on August 3 and August 9. The dosages at which these materials were applied are shown in table 1. In 1959 an experiment was conducted on commercial acreage on a larger scale and under commercial conditions. The organic phosphorus insecticides used in this experiment were malathion and Dylox® (*O,O* - dimethyl (2,2,2 - trichloro - 1 - hydroxyethylphosphonate). Three plantings were selected for their proximity and uniformity of age. The fields ranged in size from 8 to 15 acres. Pretreatment infestation counts in each field were made on July 9. Populations were determined by counting the adult beetles in eight 10-plant samples of corn; one sample taken in each corner of the field and four samples taken in a line diagonally across the middle of the field. Populations of adults are expressed in the results as adult beetles per 100 plants. Similar beetle counts were made at 3, 7 and 14 days after treatment. The 14-day sample was made July 23, the day before harvest. When the fields were harvested, samples of ears were taken at random from each field and counts made of ears infested with sap beetle larvae. Treatments were applied with the same type of high clearance sprayer that was used in the plot experiments in 1958. The treatments in both experiments were made as for corn earworm, using the usually recommended earworm formulation of 1 pound of DDT and 7 quarts of mineral oil per acre. The organic phosphorus insecticides were added to this spray.

Results.—The results obtained in 1958 in the randomized block experiment are shown in table 1. The addition of these organic phosphorus insecticides caused a reduction of injury to the ears. Table 1 shows the reduction in ear injury caused by a decrease in sap beetle infestation and not corn earworm infestation. Table 2 gives the results of the commercial scale experiments in 1959. There were considerably fewer infested ears in the fields treated with malathion and Dylox. The populations of adult beetles in the three fields varied. In the field receiving only DDT-oil emulsion there was never a large adult population. The other two fields, however, exhibited larger populations of adults during the silking period.

Discussion.—The results of experiments in both years indicated that the addition of organic phosphorus insecticides reduced infestation of ears by sap beetle larvae. The failure to obtain a valid test of significance in reduction of infested ears in 1958 can probably be attributed to the size of the treated plots. Since sap beetles move about very freely the effectiveness of insecticidal treatments may be reduced in a small area adjacent to or near an untreated plot. When a large area is treated, better results are obtained because relatively fewer beetles fly into the treated area. The results of Whitlaw *et al.* (1959) and the 1959 results reported here support this supposition. The adult population counts in the three fields treated in 1959 are confusing when compared with the ear infestation by larvae. The field receiving only DDT-oil emulsion had a small population of adults but was heavily infested with larvae. The other two fields treated with Dylox and malathion had much heavier adult populations. The reduction of the numbers of adult beetles in the Dylox- and malathion-treated fields did not reduce the adult populations to the level of the DDT-treated planting. Yet the field receiving no

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Table 1.—Results of sweet corn treatments with organic phosphorus insecticides in combination with DDT-oil emulsion for control of sap beetles. 1958.

TREATMENT		AVERAGE EARS INFESTED (%) WITH			AVERAGE UNDAMAGED EARS (%)
No. of Application of DDT-Oil Emulsion[a]	Added to DDT-Oil Emulsion (1 Qt.)	Sap Beetle Only	Sap Beetle Plus Corn Earworm	Total Sap Beetle	Angles[b]
3	25% parathion E. C.	4.6	3.3	7.9	*61.10
3	57% malathion E. C.	5.4	4.1	8.8	*65.15
3	42.2% Trithion flowable	9.4	3.9	13.4	56.01
3	25% diazinon E. C.	5.9	6.6	12.4	57.33
3	none	25.6	3.3	26.4	49.24
1	dimethoate 46% solubalized liquid	10.3	6.0	15.4	46.42
L.S.D.		n.s.	n.s.	n.s.	12.10

[a] One pound of DDT, 7 quarts of mineral oil applied in 25 gallons of water per acre.
[b] Transformed to angles for statistical treatment.

Table 2.—Pretreatment and posttreatment populations of adult sap beetles and the ear infestation by sap beetle larvae at harvest. 1959.

INSECTICIDE ADDED TO DDT-OIL EMULSION SPRAY[a]	NO. ADULT BEETLES/100 PLANTS				EARS INFESTED BY LARVAE AT HARVEST (%)
	At Time of Treatment (July 9)	After Days Shown			
		3	7	14	
1 qt. 57% malathion E. C.	51	36	43	60	8.6
2 lbs. Dylox W. P.	79	37	53	33	13.5
None	11	1	3	4	73.4

[a] One pound of DDT, 7 quarts of mineral oil applied in 25 gallons of water per acre.

organic phosphorus insecticide had the highest larval infestation. This may indicate that in this case larval infestation was not reduced altogether by reduction in numbers of adult beetles.

The addition of malathion to the corn earworm sprays resulted in the greatest reduction of larval infestation in 1958 and 1959. Whitlaw *et al.* (1959) also reported the most favorable results with malathion. These encouraging results, however, point out the need for further research with organic phosphorus insecticides for sap beetle control.

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Toxicity of BHC to Loblolly Pine Seedlings[1]

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A study was undertaken in east Texas in 1957 to determine the effectiveness of insecticidal preplanting dips in reducing damage by the pitch-eating weevil (*Pachylobius picivorus* Germ.) to loblolly pine (*Pinus taeda* L.) seedlings. The results of the control phase have already been published (Thatcher 1958); this report is concerned with the effect of BHC on test seedlings.

METHOD.—The study plots were on the Stephen F. Austin Experimental Forest, near Nacogdoches, Texas. Twelve were in areas of cutover forest with varying weevil populations, and three in an open field where no weevils were present. Prior to planting, the one-year-old seedlings were dipped in varying concentrations of wettable BHC in water suspension.[3]

In both woods and field plots, seedlings were planted in rows of 10. One row on each plot was randomly assigned one of nine treatments, involving four levels of BHC concentration, two methods of application, and an untreated check. The two methods of application were total dips in which both tops and roots of the seedlings were immersed, and top dips in which special care was taken to protect the roots from chemicals. All seedlings were examined frequently during 1957 and at the end of the 1958 season.

RESULTS.—In May 1957, phytotoxic effects of BHC total dips were evident, particularly in the heavier concentrations and in the field plots, where there was no weevil damage to mask chemical effects. By the end of the first growing season, net

Table 1.—Effect of BHC dips on planted loblolly pine seedlings.[a]

PER CENT CONCENTRATION, AND TYPE OF DIP	NET HEIGHT GROWTH[b] (FT.)						SEEDLING MORTALITY IN FIELD PLOTS[c] (NO.)		
	Woods Plots			Field Plots[c]					
	1957	1958	2-Year Total	1957	1958	2-Year Total	1957	1958	2-Year Total
Check	1.0	1.6	2.6	0.8	1.6	2.4	0	0	0
0.2 BHC									
Top	1.1	1.9	3.0	.7	1.4	2.1	0	1	1
Total	1.0	2.0	3.0	.6	1.2	1.8	1	1	2
0.5 BHC									
Top	1.0	1.7	2.7	.7	1.5	2.2	1	0	1
Total	1.0	2.0	3.0	.5	1.1	1.6	0	2	2
1.0 BHC									
Top	1.0	1.8	2.8	.7	1.8	2.5	0	2	2
Total	.7	1.5	2.2	.4	1.3	1.7	1	2	3
2.0 BHC									
Top	1.1	2.0	3.1	.7	1.4	2.1	0	2	2
Total	.6	1.4	2.0	.2	.9	1.1	12	4	16

[a] A total of 120 seedlings were used for each treatment in woods areas, 30 for each treatment in the open field.
[b] Seedlings averaged 0.4 foot in height at time of planting.
[c] Seedlings in the open-field plots were not exposed to weevils.

[1] Accepted for publication October 12, 1959.
[2] Nacogdoches Research Center, maintained by the Southern Forest Experiment Station in cooperation with Stephen F. Austin State College, Nacogdoches, Texas.
[3] Water emulsions of aldrin and heptachlor and suspensions of dieldrin were included in the weevil control test. No phytotoxic effects were noted for these chemicals.

FIG. 1.—BHC applications to roots of loblolly pine seedlings stunt growth. Seedlings in rows 1 and 2 were given total dips in 2% and 1% concentrations, respectively, of wettable BHC. Those in Row 3 were untreated.

height growth of seedlings totally dipped in 1.0% and 2.0% BHC was significantly less than that for check seedlings on all plots (table 1).

Seedling mortality owing to chemical treatment was very limited, except for total dips in the 2.0% concentrations.

Effects persisted through the second growing season (1958). Net growth of most totally dipped seedlings was lower than for those which had been top-dipped, or for the checks, and growth was least for the heaviest concentrations (fig. 1). Differences were significant in the woods plots, but not in the field. Of the 14 seedlings that died in the field plots during 1958, nine had been totally dipped, 6 of them in 1.0% or 2.0% BHC suspensions.

In a limited supplemental test, total dips in 1.0% and 2.0% BHC water emulsions produced results comparable to suspensions of the same strength.

While the specific effect of the chemical on the seedling tissues was not determined, the heavier concentrations obviously were toxic to the roots or were absorbed through them in toxic quantities. Simkover & Shenefelt (1950, 1952) found that BHC applications to the soil caused abnormal root development in Norway pine seedlings, with resultant mortality and growth retardation.

RECOMMENDATIONS.—On the basis of this study, it is recommended that BHC be employed only as a preplanting top dip for pine weevil control. A 1% or 2% concentration will provide a high degree of weevil control, but care should be taken to avoid root contamination.

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Experiments on Control of the Sugar-Beet Wireworm in Southern California Bean Fields[1]

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Experiments to determine the residual effectiveness of different soil insecticides against the sugar-beet wireworm, *Limonius*

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Table 1.—Numbers of sugar-beet wireworms trapped in 72 feet of bean or corn bait rows each spring following the application of insecticide emulsions to the soil in 1952.

Insecticide and Pounds per Acre	Year						Total
	1952	1953	1954	1955	1956	1957	
Murdy Field							
Aldrin 3	1	0	3	0	0	0	4
Heptachlor 2.5	1	1	1	1	0	0	4
DDT 10	4	0	4	2	0	0	10
Endrin 2	8	0	6	1	0	0	15
Dieldrin 2	4	1	15	2	0	0	21
Chlordane 5	6	4	9	3	0	0	22
Check	32	33	142	50	18	8	273
L.S.D. at 5% level[a]	b	2	b	b	b	b	b
Drew Field							
Endrin 2.3	1	0	—	0	1	1	3
Toxaphene 15	7	0	—	1	0	0	8
Dieldrin 2.2	12	0	—	2	1	0	15
DDT 12	25	0	—	1	4	7	37
Chlordane 4.6	23	1	—	22	45	25	116
Aldrin 2.3	40	2	—	31	13	41	127
Heptachlor 1.25	49	0	—	25	45	29	148
Check	44	1	—	48	84	70	247
L.S.D. at 5% level[a]	31	b	—	22	27	30	76

[a] Untreated checks not included.
[b] Not significant according to *F* test.

californicus Mann., were begun in two fields in southern California in 1952 and continued through 1957.

The insecticides were applied as emulsions to the soil surface with a power sprayer at the rate of 35 gallons per acre on March 31 and June 16, 1952, in the Murdy and Drew fields, respectively. Immediately after application they were thoroughly mixed with the soil by disking 7 to 8 inches deep and the soil was firmed by dragging. The wireworm population was determined each year by planting untreated bait beans or corn in rows before planting the main crop, except in the Drew field in 1953, when the baits were planted 3 weeks after the main crop. Four to eight soil samples (4 by 4 by 24–36 inches) were taken in the bait rows of each plot 7 days later, and each sample was sifted with a hand sifter equipped with a 12-mesh screen. Because of the variation in the size of the samples, the data given in table 1 have been corrected to 72 feet of row. Bait beans were planted in the different years between March 29 and May 16 in the Murdy field, and between May 1 and July 27 in the Drew field.

Murdy Field.—This was a field of adobe soil in Orange County, where for several years beans had been severely damaged by wireworms. During the experiment it was planted each year to lima beans and kept fallow during the winter months.

The experimental plots were 50 by 175 feet and arranged in six randomized-block replicates. In 1952 all insecticides significantly reduced the wireworm populations within 30 days (table 1). They continued to give excellent control in 1953, and good stands of beans were obtained in all treated plots. In 1953 and thereafter, the entire field (both treated and untreated plots) was planted by the grower with seed coated at the warehouse with lindane slurry at the rate of $\frac{1}{3}$ ounce per 100 pounds, but judging by the buildup of wireworm populations in the check plots, the seed treatment did not appreciably affect the results. However, the bait beans used in obtaining wireworm population data were untreated.

All treatments gave excellent control, especially in 1954 when the infestation increased to 1.8 wireworms per foot of row in the untreated plots. In the last 3 years very few wireworms were recovered in the treated plots, but moderate infestations were present in the untreated plots. Best results over the 6-year period were obtained with aldrin and heptachlor. Even when the untreated plots were planted with lindane-treated seed, moderate to high populations prevailed and some damage occurred each year, especially in the more heavily infested plots.

Drew Field.—This field was of sandy loam, and was located in Riverside County. It was planted intermittently to blackeye beans and winter crops of barley. The plots were 30 by 112 feet and were arranged in five randomized-block replicates. Of the insecticides tested in 1952, only endrin, toxaphene, and dieldrin gave significant reductions in wireworm populations in the 35-day period following application of the materials. Owing to a miscalculation, only one-half the required dosage of heptachlor, or $1\frac{1}{4}$ pounds per acre, was applied in the soil. This miscalculation was unfortunate, as heptachlor at twice this dosage has generally given satisfactory control of wireworms. In 1953 the cull baits used to attract worms were planted between rows of the regular crop of blackeye beans 3 weeks *after* planting, which probably accounts for the absence of wireworms in the bait rows of the untreated plots, because the worms had no doubt already been attracted to the previously planted rows of the main crop. Also, very few wireworms were trapped in the bait rows of the treated plots in 1953 for the same reason. However, since no injury to the stand of blackeye beans was noted, it appeared that all the insecticides had given control a year after treatment. No population data were obtained in 1954 because the field was planted to barley. Endrin, toxaphene, and dieldrin continued effective through 1955, 1956, and 1957. DDT was ineffective in 1952, but very effective in 1953 through 1957.

Discussion.—Aldrin at 2 to 3 pounds per acre has been inconsistent in control of wireworms, and this fact is confirmed by earlier results (Stone & Foley 1953, Woodworth & Lane 1957). In the Murdy field aldrin gave excellent control, but was ineffective in the Drew field. Toxaphene at 15 pounds per acre was highly effective in the Drew field and should be given consideration for future tests. In the Murdy field DDT gave significant control of wireworms 30 days after application, which is quick action for this insecticide. Chlordane was erratic, that is, fairly effective in one field and ineffective in the other. Of the insecticides tested, the most consistent were dieldrin, DDT, and endrin, all giving excellent control of wireworms for 6 years.

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The Shallot Aphid in the Pacific Northwest[1]

Charles F. Doucette, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

In the last few years the shallot aphid (*Myzus ascalonicus* Doncaster) has been found in Easter lilies in several locations in the Pacific Northwest. The writer first encountered it in a commercial greenhouse at Sumner, Washington, in February 1955.[2] Later that spring it was found near Arcata, California, on lily plants growing in a pile of bulblets and cull bulbs left from cleaning and grading operations the previous fall. In January 1956 infestations were seen on Easter lilies in a greenhouse at Portland, Oregon, and have since been noted several times in greenhouses during the forcing seasons. In May, 1956 a moderate infestation was found on lilies in a cull pile at Smith River, California, and in October a very heavy infestation was observed at Harbor, Oregon. The aphid has been collected on Easter lilies in southern Oregon and northern California a number of times each season since 1956. Its occurrence in cultivated plantings has been irregular, because of persistent control procedures used by the growers, but it can usually be found on plants on the rubbish and cull piles. These are probably an important means of continuance of the population in the lily-producing area, particularly

[1] Accepted for publication October 30, 1959.
[2] Determined by Louise M. Russell, Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture.

in the late fall and early winter, when the plants are not up in the commercial fields.

These findings on Easter lilies instigated a survey of the available information about this aphid. The original description by Doncaster (1946) was based on specimens collected in England in 1941. However, the earliest collection was on April 24, 1940, at Evanston, Wyoming, on onion sets sent from Detroit, Michigan (Fronk 1955). MacGillivray (1954) reported its presence on carrots at Chilliwack, British Columbia, in October 1947 and on chrysanthemum plants held in a potato storage at Fredericton, New Brunswick, in March 1954.

In 1955 the aphid was recorded as a pest of strawberry for the first time in North America. It caused serious losses on farms in southern Vancouver Island and in the lower Fraser Valley, British Columbia (Andison 1956). In 1958 P. M. Eide and E. P. Breakey found it on strawberries in western Washington and R. A. Rosenstiel found it in the Willamette Valley in Oregon (U. S. Plant Pest Control Division 1958). Its behavior on strawberry in the Pacific Northwest corresponded closely to observations in England by Dicker (1950). In all areas the aphids left the strawberry plants in May or early June and returned in October. Severe injury to strawberry has been associated with mild winter and early spring weather. Other plants on which this aphid has been found in North America are chickweed in several localities, viola and pansy at Sumner, Washington.

According to the early European accounts nothing was known about natural hosts during the summer period. In laboratories, colonies were maintained the year around on various plants, particularly the shallot. In the Pacific Northwest its occurrence on carrot in large numbers in the fall indicates that it was probably on that host during the summer. It has been collected several times on lilies during the summer months. Infestation on pansy plants was noted in July 1958 at Sumner, Washington. Heavy infestation was observed on chickweed in an Easter lily field near Arcata, California, on September 20, 1956. Semal (1957) reports collections on *Stellaria*, *Capsella*, and shallots between July and September. Since its description the aphid has been reported on numerous host plants in many European countries.

The greenhouse infestations on Easter lilies do not necessarily indicate outside infestations. Apparently the aphids may be carried with the bulbs, and the greenhouse infestations seemed to be chiefly on individual plants but spreading slowly to neighboring plants. A tendency to be carried on plant material in commerce is indicated by records of interceptions at U. S. ports of entry. In the lists issued by the Plant Quarantine Division of the U. S. Department of Agriculture the shallot aphid is recorded as having been intercepted on onion in 1949; on shallot, gladiolus, and tiger lily in 1950; on chicory, gladiolus, and ivy in 1954; on shallot in 1957; on lettuce and tulip in 1958.

It is evident from European studies that the shallot aphid is a very efficient vector of viruses. Heinze (1952) reports it as transmitting onion yellow dwarf and turnip mosaic. Doncaster & Kassanis (1946) report successful transmission of beet yellows, henbane mosaic virus, and cucumber mosaic virus (Cucumis virus 1 Doolittle). Kassanis (1947) states that this aphid transmitted dandelion yellow mosaic to lettuce, which *Myzus persicae* (Sulz.) did not, and also the lettuce mosaic virus. Semal (1957) had successful transfer of beet mosaic virus, beet yellows, and cucumber mosaic virus, but not of potato virus Y. Badami (1958) used it successfully in transmitting certain strains of cucumber mosaic virus. So far as is known, there are no records of virus transmission by this aphid in North America.

Essig's (1958) use of the name "shallot aphid" for *Micromyzus formosanus* Takahashi has caused some confusion. It has resulted in at least one statement that *Myzus ascalonicus* was of Formosan origin. Essig was in error in stating that the paper of Doncaster & Kassanis (1946) referred to *formosanus*, and his reference to Great Britain in the distribution of *formosanus* is probably also in error. *M. formosanus* is almost black and the cloudy veined wings of the alates are characteristic of the genus. *Myzus ascalonicus* is uniformly pale brown, or greenish brown, or dull yellow, sometimes with irregular dark olive-green markings on the abdomen; the dorsum is strongly convex and shining. Superficially *ascalonicus* resembles *persicae*. The cornicles of *ascalonicus* are slender and distinctly swollen on the apical half, and are smaller in relation to the rest of the insect than those of *persicae*. Also the antennal tubercles are more convergent than in *persicae*.

It is expected that further investigations will reveal a much wider distribution of this aphid in North America. Its wide host range in Europe and its recognized efficiency in virus transmission indicate that this aphid can be a serious threat to many crops in North America. In view of these potentialities a more active search for infestations and tests to determine the aphid's ability to transmit virus diseases seem desirable.

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A Nematode Parasite of the Alfalfa Weevil (*Hypera postica* (Gyll.))[1]

GEORGE O. POINAR, JR. and GEORGE G. GYRISCO[2]

On June 20, 1959, several sluggish, affected alfalfa weevil larvae were noted among others that had been collected that day

[1] Accepted for publication October 26, 1959.

[2] The authors wish to acknowledge the assistance of Donald Adams in the field investigations.

Thanks is given to A. L. Taylor, U.S.D.A., who identified these nematodes to family.

FIG. 1.—A nematode emerging from a fourth-instar alfalfa weevil larva. The other nematodes in the picture emerged from similar larvae. (Photo by J. A. Adams)

and the previous day from an alfalfa field in Dutchess County, New York. These were dissected and found to be parasitized by nematodes. Later that same day, 24 more larvae were collected from sweeping the same field, and six were found to be parasitized by nematodes.

On June 21, 12 out of 42 fourth-instar larvae collected from the same field were found to be parasitized. From one to three nematodes were found in a single larva. The length of the nematodes varied from 3 to 10 centimeters. Only fourth-instar larvae were found parasitized by the nematodes (fig. 1).

A survey of other alfalfa fields in the southern part of Dutchess County on June 24 and July 3 showed that three other fields contained parasitized larvae. An examination of preserved larvae collected earlier in 1959 from control experiments showed that parasitized larvae were also present in Ulster and Orange Counties, which are adjacent to Dutchess County.

Alfalfa weevil larvae that are parasitized by nematodes are wrinkled and bulky in appearance and usually are a little larger than normal larvae. They are sluggish and respond less to probing than normal larvae.

Nematode specimens sent to Washington, D. C. were identified as belonging to the family Mermithidae.

BOOK REVIEWS

SUBTROPICAL FRUIT PESTS, by WALTER EBELING. University of California, Division of Agricultural Sciences, 1959, 436 pp. This book may be ordered from the University of California, Agricultural Publications, 207 University Hall, Berkeley 4, California. Price, $7.00.

Dr. Ebeling's revision of his book on subtropical fruit pests is an extremely useful volume which should be present in all personal and general scientific libraries. Major emphasis is on insect and mite pests of fruit and nut crops grown in subtropical areas of the United States, with citrus pests receiving especially thorough treatment. Several color plates and the many other illustrations are attractive features. The extensive bibliography provides a convenient reference source for those who may wish to develop more detailed knowledge about certain subjects.

Despite its comprehensive title, which suggests consideration of all categories of subtropical fruit pests, the book is concerned primarily with entomology in the presently accepted sense and only brief mention is made of a few other animals which damage fruit crops. The information on control methods and materials in Part I has general application and interest. The initial chapter on legislative and organizational controls deals with a subject not usually discussed in references of this type.

L. D. CHRISTENSON
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GRASSLANDS. Publication No. 53. HOWARD B. SPRAGUE, Editor. American Association for the Advancement of Science. Washington, D. C. 1959. 6×9, xv+424 pp., Illus. $9.00.

The book is the outgrowth of a symposium on various aspects of grassland problems held at the New York meeting of the AAAS in December 1956. Forty-four specialists in different fields related to grassland agriculture contributed to the 37 papers included in the volume. Because of the diversity of subject matter and the large number of authors an adequate review of all of the articles is almost impossible.

The papers are grouped under eight major headings. The first group includes papers on the interrelations of the different sciences that support grassland research. A second, on forage production in the temperate humid region, includes papers on new varieties and on disease and insect control. Other sections cover the engineering aspects of forage production, forage utilization, nutritive significance of forages, grassland climatology, ecology, and range management. An index adds considerably to the usefulness of the volume.

The substantial increase in grassland farming in the past decade and its importance in our national economy make this volume a timely one. The many developments in grassland research and practice reviewed in the book should make it a useful reference to scientists, engineers, and administrators, who, though concerned with certain specific problems of grassland agriculture, may wish to survey other aspects of this important field.

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Entomology Research Division
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OBITUARIES

Frederick Zeller Hartzell
1879-1958

Following several years of steadily declining health, Frederick Zeller Hartzell, Emeritus Professor of Entomology, died June 13, 1958, at Geneva, New York. He was in his 78th year. He had been a member of the staff of the New York State Agricultural Experiment Station since 1909, retiring in 1948.

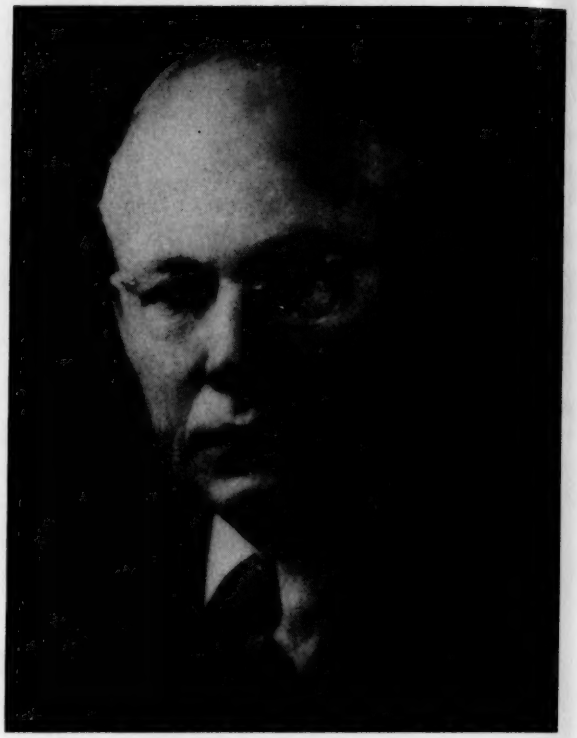
Professor Hartzell was born on a farm near Easton, Pennsylvania, December 11, 1879. He received a classical education at Lafayette College where, in 1905, he received the AB degree. During his junior year at Lafayette he became afflicted with tuberculosis. While his case was mild and responded successfully to treatment, he decided that with such a susceptibility it would be best to prepare for a career in a field that would keep him outdoors as much as possible. Also, he saw in this plan an opportunity to pursue his newly awakened interest in science.

Consistent with these earlier decisions, in 1906 he accepted a position as Field Assistant in the Zoology Division of the Pennsylvania Department of Agriculture. His first assignment was scouting for the San Jose scale. This much-feared pest of tree fruits had only recently been introduced into Pennsylvania. Hartzell's mission was to seek out, inspect, and record the scale-status of host trees wherever they might occur in the western half of the State. This quest took him into many remote and little-traveled districts. Much of the time he relied on his own two legs to get from one farm to the next, although, on some expeditions, he made use of a bicycle.

In 1907 he was assigned to a Federal-State field research station at North East, Pennsylvania. Here he came under the influence of the federal entomologist, A. G. Hammar, who convinced him he needed more formal training in entomology if he was to fulfill his plan of a career in this field. This led him to Cornell University where, in 1909, he was granted the MA degree. That same year he was appointed to the staff of the New York State Agricultural Experiment Station as Assistant Entomologist and was assigned to the Station's Vineyard Laboratory at Fredonia which remained his headquarters until 1928 when he moved to Geneva. Professor Hartzell attained his full professorship in 1938. For several months in 1948, following the death of Doctor Hugh Glasgow, he served as acting head of the Station's Department of Entomology. He retired December 31, 1948.

Professor Hartzell was well and favorably known in various agricultural circles of the State. Best known to fruit growers, he was held in especially high esteem by the grape growers of Chatauqua and Erie Counties where, during the first two decades of his career, he worked so diligently and effectively in their interest. Much of our present knowledge of the biology, ecology, and control of the many pests of grape, pear, apple, and cherry under New York conditions is based on Professor Hartzell's research. He also was active in the introduction and development of certain insecticides, notably the dinitro compounds and the tar oils. In the area of contributions to his profession, Professor Hartzell will possibly be remembered best for his pioneering in the application of statistical and biometrical principles to entomological field experimentation. In all, he authored some 95 technical or semitechnical papers.

In addition to Entomology, Professor Hartzell was well informed in the fields of Ecology, Ornithology, Biometry, Geology, Astronomy, Botany, Meterology, and in several branches of Mathematics. It can be said he attained professional competence in the first four fields. However, of all titles the one that perhaps fits him best was that of Naturalist. Professor Hartzell was a member of the following scientific societies: the American Association for the Advancement of Science (fellow), Entomological Society of America, American Association of Econonic Entomologists, American Chemical Society, Ecological Society of America, and American Statistical Association.



Frederick Zeller Hartzell

Professor Hartzell was highly respected by his colleagues and associates for the breadth and depth of his knowledge. In venturing into fields outside their own specialization, many found it quicker and easier to "ask Hartzell" for the information they sought than to dig it out themselves. His knowledge was truly encyclopedic. An inveterate reader, his curiosity knew no bounds. Professor Harzell was at his best in the role of instructor and counselor. Many can look back with gratitude to the help they received as graduate students or as new staff members from this patient, kindly man. His aid was constantly being sought in such diverse fields as writing, instrumentation, the layout and preparation of graphs, design of field experiments, the statistical analyses of data, and others.

Modest and unpretentious, Professor Hartzell was invariably courteous and thoughtful of others. There was no malice in him. It was rare indeed to hear him speak unfavorably of anyone—of anyone, that is, except himself. His intimates derived much amusement from this habit of self-criticism. Professor Hartzell allowed himself few indulgences. One of these was the acquisition of books; his personal library, composed largely of treatises on scientific subjects, exceeded a thousand volumes. He also received pleasure from the possession of such equipment as cameras and field glasses, of which he owned a half dozen each. These he put to good use in the frequent excursions he made afield to photograph various objects and scenes of interest and to view birds.

Professor Hartzell always exhibited pleasure and enthusiasm for his work and interests. One gained the impression he found living in this world a great and exciting adventure; his interest in learning more and still more about it all never flagged.

Professor Hartzell married Edith M. Rupp in 1910. She died in 1933. He is survived by a brother, Dr. Albert Hartzell of the

Boyce Thompson Institute at Yonkers, New York; by a sister, Mrs. Martha W. Clagett, a half-brother, Wilson E. Hartzell, and a half-sister, Mrs. Verna Krissinger, all of Lebanon, Pennsylvania; and by several nieces and nephews.

In Professor Hartzell's passing the world has lost a true scholar and gentleman.

P. J. CHAPMAN

Howard Herman Jewett
1884-1959

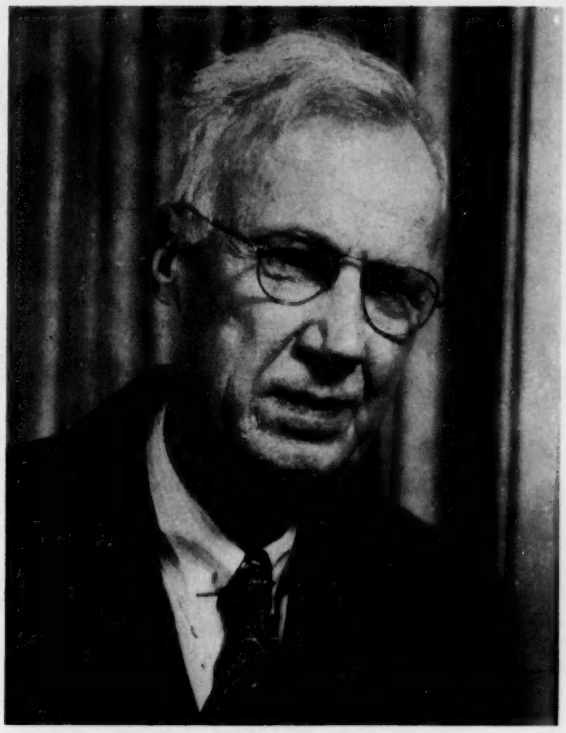
Howard Herman Jewett was born October 25, 1884, at Celina, Ohio. Here his boyhood was spent and here he attended three years of high school. This was followed by one year in the Ohio Wesleyan Academy. He entered Ohio Wesleyan University in the fall of 1903 and received the Bachelor of Arts degree in 1907.

During 1907–08 he taught in the Urbana (Ohio) High School and in the Albion (Michigan) High School the folllwing year (1908–09). In 1910 he received the Master of Arts degree from Ohio State University. This was followed by one year as Assistant Botanist on the staff of Maryland Agricultural College. He came to the University of Kentucky Agricultural Experiment Station on June 1, 1911, as Assistant Research Entomologist and remained here for the rest of his scientific career. He was promoted to Associate Research Entomologist on July, 1 1946, and retained this title until assuming change-of-work status on November 1, 1954.

Mr. Jewett, during his Kentucky career, worked in the general field of economic entomology but early began to devote considerable time to the study of tobacco insect pests. Although he worked on numerous problems, he was best known for his tobacco insect work. His published bibliography lists approximately 50 bulletins, circulars and papers on insects and their control. These titles show the diversity of his work.

He held membership in the following organizations: Entomological Society of America, American Association for the Advancement of Science (Fellow, 1928), Sigma Xi (President of the Kentucky Chapter, 1943–44), Kentucky Archaeology Society (President, 1943–44), Kentucky State Beekeepers Association (President, 1943), Kentucky Research Club and Kentucky Academy of Science. In 1949 he was President of the Tobacco Insect Council, an industry-wide group of entomologists working in the field of tobacco insect control.

Mr. Jewett had several hobbies which furnished him relaxation and enjoyment. He was an amateur archaeologist and derived much pleasure from his excursions to nearby areas in search of materials of interest in this field. He collected stamps and his collection was a proud possession. Mr. Jewett took pride in his garden, both vegetables and flowers, and was successful in these endeavours. He was a quiet individual, almost shy at times. He was a member of the Presbyterian Church.



Howard Herman Jewett

Mr. Jewett passed away in the Good Samaritan Hospital, Lexington, on April 2, 1959. He is survived by his wife, Mary LaRue Jewett whom he married in 1913. Interment was in the Lexington Cemetery on April 6, 1959.

LEE H. TOWNSEND

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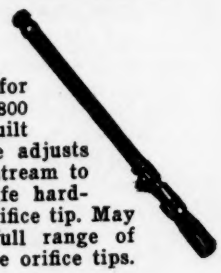

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